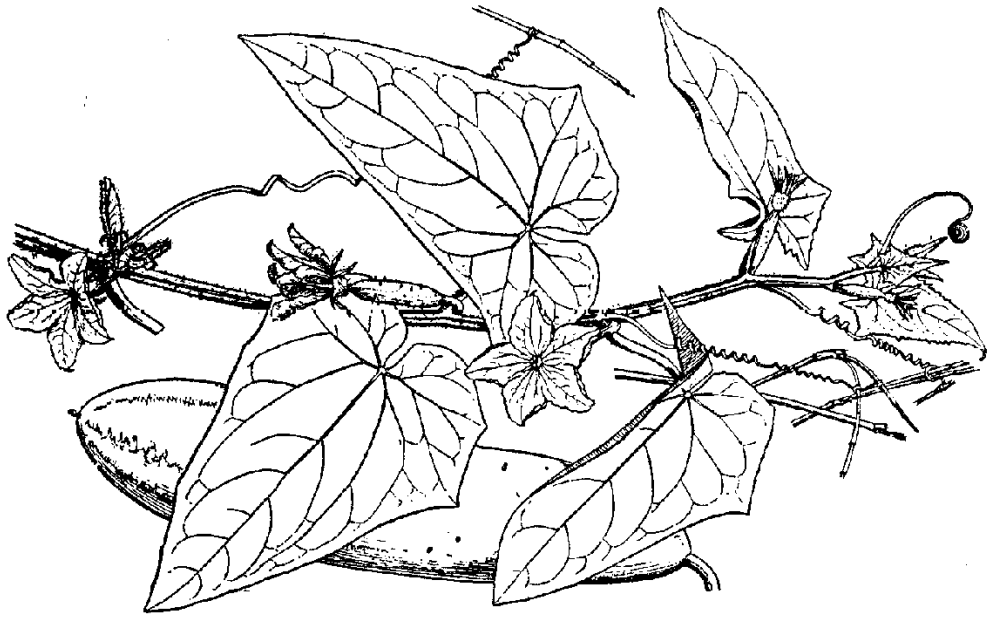
Cucurbit Genetics Cooperative

2001



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Cucurbit *Genetics* Cooperative

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The **Cucurbit Genetics Cooperative** (CGC) was organized in 1977 to develop and advance the genetics of economically important cucurbits. Membership to CGC is voluntary and open to individuals who have an interest in cucurbit genetics and breeding. CGC membership is on a biennial basis. For more information on CGC and its membership rates, visit our website (*http://ars-genome.cornell.edu/cgc/*) or contact Tim Ng at (301) 405-4345 or *tn5@umail.umd.edu*.

CGC Reports are issued on an annual basis. The Reports include articles submitted by CGC members for the use of CGC members. None of the information in the annual report may be used in publications without the consent of the respective authors for a period of five years.

ISSN 1064-5594

2000 CGC Business Meeting

Timothy J Ng, CGC Chair
University of Maryland, USA

The 24th annual CGC Business Meeting was held on 23 July 2000 at the Coronado Springs Hotel in Orlando, Florida, in conjunction with the 2000 International Conference of the American Society for Horticultural Science (ASHS). Seventeen members and other interested individuals attended.

After introductions around the room, Tim Ng provided a brief summary of the history of the CGC Reports, as well as a financial status update. He said he was starting to consider CGC Report No. 23 as "haunted," in that strange events were combining to delay its publication, but that it would be completed and printed by August 2000. Part of the delay was due to working out the logistics of including the proceedings from the 1st International Oil Pumpkin Conference, which was held in Austria in 1999.

The Call for Papers for CGC 24 (2001) is scheduled for mailing in October 2000, with a deadline date of 28 February 2001. As has been the practice in recent years, manuscripts submitted after the deadline date may still be considered for inclusion in the Report -- subject to the time constraints of the Coordinating Committee members and the Chair.

Tim next indicated that the CGC website was proceeding slowly but surely. A recent addition to the web CGC Reports page was the indication of the availability of back issues and also whether a given report is available on the web.

Jim McCreight provided a brief report on the recently completed *Cucurbitaceae 2000* conference in Israel, and also mentioned that the *2nd ISHS International Conference on Cucurbits* was scheduled for Japan sometime in 2001. However, information was scarce on the latter conference and no one present at the CGC meeting had attended the 1st ISHS Cucurbit conference, so the Chair agreed to track down more information on the Japanese meeting.

Because of increasing publication and postage costs, it was announced that membership rates would increase to $20 per biennium (surface rate) beginning in 2002. The increase would be phased in for individuals renewing in 2001. However, thanks to an arrangement with the University of Maryland, CGC members would now be allowed to charge their membership to their credit card;

Comments…….

From the CGC Coordinating Committee: The Call for Papers for the 2002 Report (CGC Report No. 25) will be mailed in October 2001. Papers should be submitted to the respective Coordinating Committee members by 31 March 2002, although late submissions may be considered if received prior to our processing deadline. The Report will be published by July 2002. As always, we are eager to hear from CGC members regarding our current activities and future direction of CGC.

From the CGC Gene List Committee: Lists of known genes for the Cucurbitaceae have been published previously in HortScience and in reports of the Cucurbit Genetics Cooperative. CGC is currently publishing complete lists of known genes for cucumber (*Cucumis sativus*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), and *Cucurbita* spp. on a rotating basis.
It is hoped that scientists will consult these lists as well as the rules of gene nomenclature for the Cucurbitaceae before selecting a gene name and symbol. Thus, inadvertent duplication of gene names and symbols will be prevented. The rules of gene nomenclature (published in each CGC Report) were adopted in order to provide guidelines for the naming and symbolizing of genes previously reported and those which will be reported in the future. Scientists are urged to contact members of the Gene List Committee regarding questions in interpreting the nomenclature rules and in naming and symbolizing new genes.

From the CGC Gene Curators: CGC has appointed curators for the four major cultivated crops: cucumber, melon, watermelon and *Cucurbita* spp. Curators are responsible for collecting, maintaining, and distributing upon request stocks of know marker genes. CGC members are requested to forward samples of currently held gene stocks to the respective Curator.

this would be a welcome benefit to many of the international members who previously needed to exchange currency for their membership.

Watermelons were the next topic of conversation, as a Gene Curator was needed for the watermelon monogenic stocks, and also a new CGC Coordinating Committee member was needed to replace Dennis Ray. The Gene Curator decision was deferred, but a nomination committee consisting of Jim McCreight, Jack Staub and Tom Williams nominated Todd Wehner for the Coordinating Committee position, and he subsequently accepted.

Under "new business," Tim Ng indicated that he was planning to step down as CGC Chair in 2002, following publication of CGC Report No. 25. This would mark his 15th year chairing CGC, and although he would like to continue, his job responsibilities at the University of Maryland had changed so much in recent years that he is able to spend very little time on horticultural matters. Over the next two years he will work with the CGC membership to identify a successor, and also to determine whether the Chair position should more logically be divided up among three individuals to spread the workload.

2001 CGC Business Meeting

Timothy J Ng, CGC Chair
University of Maryland, USA

The 25th annual CGC Business Meeting was held on 22 July 2001 in the Sacramento Convention Center in Sacramento, California, in conjunction with the 2001 International Conference of the American Society for Horticultural Science. Twenty members and other interested individuals attended.

After introductions around the room, Tim Ng introduced Yasutaka "Taka" Kubo, one of the organizers of the 2nd International Symposium on Cucurbits. Taka passed out announcements of the conference and reported that it was scheduled for 28 September through 1 October 2001. Taka mentioned that registration was now open at the conference website, and encouraged everyone to consider attending.

Tim next reported that CGC 24 was slightly delayed because of some late arriving manuscripts, but was scheduled to go to the printers within the next week. He estimated that it would be over 160 pages, and about equivalent in size to the previous year's report. The Call for Papers for CGC 25 is scheduled to go out in October 2001, with a deadline in February or March.

CGC 25 (2002) will mark the *"Silver Anniversary"* issue of the CGC Report, and Tim indicated he would try to have a special cover prepared for the report. Options include a silver cover, or the possibility of a "scratch'n'sniff" cover with cucurbit-related odors. He also mentioned that CGC does not have an "official" logo, and is considering a CGC logo design contest, with the logo to be displayed on the cover of CGC 25. This contest may be announced in conjunction with the "Call for Papers" solicitation in October.

CGC Coordinating Committee member Linda Wessel-Beaver raised the issue that research reports tended to be longer than in the early years of CGC, often 5-6 pages in length, and was concerned whether she should edit her section more severely to reduce the manuscript size or request that the authors send them to a refereed journal. Also, the types of acceptable papers were discussed. (e.g., should only research reports be allowed, or are literature reviews on relevant topics also acceptable?) In addition, a discussion ensued as to whether CGC page limits, currently 2-3 pages, should be strictly enforced. Tim indicated that the CGC budget is able to (barely) cover the current size of the CGC report, and it was ultimately decided to allow flexibility in the size and acceptability for the research reports.

A page detailing upcoming meetings of interest to cucurbit researchers was passed out, and Tim mentioned that the proceedings from recent cucurbit conferences were now available through either ASHS or ISHS presses. (Editor's note: see later item *"News and Comment."*)

Next, the CGC website was discussed. Tim indicated that he felt a need to change the "look and feel" of the main CGC page, which is nearly 5 years old at this point, and also to make the site easier to navigate. Todd Wehner suggested that perhaps the main page could look similar to the inside cover of the CGC Report. Tim also cited the need to consolidate the main *Cucurbita* spp. on-line gene list from 1992 with the two subsequent updates (1996 and 2000). Rebecca Brown volunteered to consolidate these lists, and Tim indicated he would email the electronic files to her to facilitate this. It was also

unclear whether Dick Robinson would be continuing as the Gene List Committee member for *Cucurbita* upon his retirement, and Tim indicated he would check into this.

With the impending resignation of Tim Ng as Chair of CGC, the issue of a new Chair was discussed. Tim indicated that the job is much larger than it was 14 years ago, particularly with in-house preparation of the CGC Report, email communications, international currency exchange(s), and website development. He thought that perhaps the duties might be spread over three positions, such as Chair (i.e., Secretary/Treasurer), Editor (CGC Report) and Webmaster. Tim indicated he would be happy to help with the transition, and even possibly assume one of these positions. Should this re-organization occur, it will require a change to the CGC By-Laws, necessitating a mail/email ballot among the membership. Todd Wehner suggested that, with restructuring and changing the By-Laws, perhaps we ought to change the Coordinating Committee terms to five years instead of ten, and involve more of the younger CGC members in CGC activities. Although it was anticipated the overall restructuring would take some time, it was felt that a final structure could be agreed upon by either the 2002 ASHS/ISHS meeting or the *Cucurbitaceae 2002* meeting in Florida.

Under "New Business," Jim Myers raised the issue of whether CGC would be interested in coordinating an effort to seek funding for a cucurbit genome project. Tim indicated that an early attempt was started this year with the USDA IFAFS solicitation, but that many of the researchers were concerned over the low success rates for proposals to the IFAFS genome section (only 8% in 2000). Ted Carey mentioned that he had received an IFAFS grant recently in a non-genome area, but that the success rate among proposals in that area was also very low. Jim McCreight indicated that USDA National Plant Germplasm System funds were extremely limited and nowhere near what would be needed for a genome effort. Tim said that there appeared to be only two potential sponsors for a project the size and scope of a proposed cucurbit genome effort, NSF and USDA (e.g., NRI and IFAFS), and that NSF had provided plant genome funding in the past as long as the projects didn't appear to emphasize "crop" plants. As NSF is in the Washington D.C. area, Tim indicated he would try to either phone or meet with an appropriate NSF program director to discuss the prospects of a project like this.

2001 Meeting of the Watermelon Research and Development Working Group

Benny D. Bruton, WRDWG Chair
USDA ARS, Lane, Oklahoma 74555

The 21st Annual Meeting of the Watermelon Research and Development Working Group (WRDWG) was held on Sunday, 28 January 2001, in Fort Worth, Texas. The meeting was held at the Worthington Hotel in conjunction with The Southern Association of Agricultural Scientists (S.A.A.S.) and the Southern Region of the American Society for Horticultural Sciences (SR: ASHS). There was an excellent program this year with an attendance of approximately 75 people.

Research updates included (1) Don Maynard, Horticulturalist, University of Florida, Bradenton, FL, "*Review of the Florida Statewide Watermelon Trials*"; (2) Frank Dainello, Extension Horticulturalist, Texas A&M University, College Station, TX, "*Review of the Texas Statewide Watermelon Trials*"; (3) Amnon Levi, Research Geneticist, USDA-ARS, Charleston, SC, "*Construction of an Initial Genetic Linkage Map for Watermelon Using a Population that Segregates for Fusarium Wilt Resistance*"; (4) Penelope Perkins-Veazie, Plant Physiologist, USDA-ARS, Lane, OK, "*Lycopene Content of Watermelon and Correlation with Colorimetric Measurements*"; (5) David Bender, Horticulturalist, Texas A&M University, Lubbock, TX, "*Successful Production of Triploid Watermelon Transplants*"; (6) Dan Egel, Plant Pathologist, Purdue, Vincennes, IN, "*Mature Watermelon Vine Decline: a Disease of Unknown Etiology in Southwestern Indiana*"; (7) Maciej Biernacki, Horticulturalist, Oklahoma State University, Lane, OK, "*Image-based Quantitative Assessment of Foliar and Soilborne Diseases*"; (8) Sam Pair, Research Entomologist, USDA-ARS, Lane, OK, "*Squash Bug Transmission of the Yellow Vine Bacterium and Potential for Using Trap Crops for Control*"; (9) Angela Davis, Research Geneticist, USDA-ARS, Lane, OK, "*Evaluation of Yellow Vine Resistance in Watermelon PI's*"; (10) George Boyhan, David Langston, Pamela Lewis, and Donna Linton, Extension Horticulturalist, University of

Georgia, Statesboro, GA, "*Evaluation of the USDA Watermelon Germplasm Collection for Resistance to Fusarium Wilt and Root Knot Nematode*"; (11) Todd Wehner, Horticulturalist, North Carolina State University, Raleigh, NC, "*Resistance to Gummy Stem Blight in Watermelon*"; (12) Benny Bruton, Research Plant Pathologist, USDA-ARS, Lane, OK, "*Powdery Mildew on Watermelon: Outlook for the Future*"; (13) Gerald Holmes and Jonathan Schultheis, North Carolina State University, Raleigh, NC, "*Susceptibility of Watermelon Cultigens to Ozone Injury in North Carolina*"; and (14) Jim Shrefler, Extension Horticulturalist, Oklahoma State University, Lane, OK, "*Watermelon Tolerance to Halosulfuron Herbicide and Weed Control Efficacy*."

Todd Wehner also presented an update on the status of watermelon Fusarium wilt differentials, and Diana Musto (Research Associate, Orlando, Florida) provided an update from the National Watermelon Promotion Board.

Benny Bruton next spoke on the status of watermelon Plant Introductions. He mentioned that Robert Jarret (Curator, USDA-ARS, Plant Genetic Resources Unit, Griffin, GA) has tried to get help in establishing a core collection for a long time, and that they are now in the process of selecting the core collection and hopefully will have something finalized this year. The Cucurbit Crop Germplasm Committee and the Cucurbit Genetics Cooperative Coordinating Committee will be consulted for final approval of the core collection. Robert Jarret then presented the "Curator's Report on Watermelon Germplasm."

A motion was made and carried that WRDWG meet for two half-day sessions in 2002. Provided that Benny Bruton can get the slots for Saturday and Sunday afternoon, WRDWG will have two half-day sessions. The meeting for 2002 will be held 2-3 February 2002 in Orlando, Florida.

Glenn Price of Sugar Creek Seed Inc. in Hinton, Oklahoma, generously provided refreshments for the group this year.

Cucurbit Crop Germplasm Committee (CCGC)

Call for Germplasm Evaluation Proposals - FY2002

James D. McCreight
Chair, CCGC

Now is the time to write (or prompt your colleagues to write) and submit germplasm evaluation proposals for FY2002. Please note that the U.S. National Plant Germplasm System (NPGS) will not be able to fund all of the proposals submitted, so please be very realistic in your request for funds.

All proposals will be evaluated on the national need for evaluation data, the likelihood of success, and the likelihood that the data will be entered into the *Germplasm Resources Information Network* (GRIN) and shared with the user community. Proposals should be succinct. They must be for germplasm evaluation *per se*, not for selection or improvement.

Contact the CCGC Chair for details including proposal guidelines and timeline for submission and review. The deadline for original submission is 31 August 2001.

2nd International Symposium on Cucurbits

28 September – 1 October 2001
Epochal, Tsukuba, Japan

The Cucurbit Working Group of the International Society for Horticultural Science (ISHS) is hosting the 2nd International Symposium on Cucurbits. The Symposium is intended for researchers, scientist, students and professionals who are interested in any aspects of cucurbits. The aim of the symposium is to provide the opportunity for researchers and scientists from various countries and regions of the world to discuss their work and to exchange ideas on all aspects of cucurbit science, technology and industry.

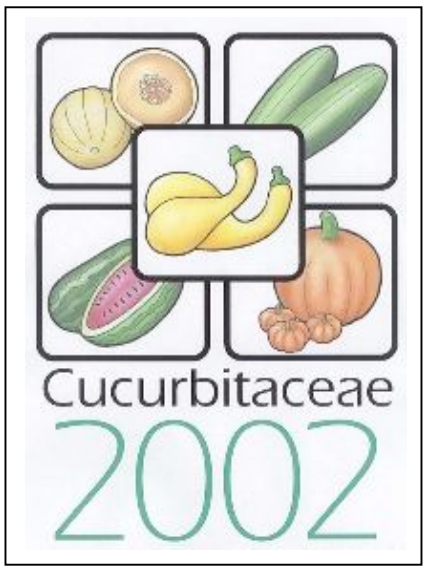
The scientific program will include country reports by invited speakers from major growing areas, presentation of papers on selected topoics by invited speakers, and research papers by oral presentations or posters. Selected topics include: development and physiology; production technology; genetics and breeding; biotechnology; pests and diseases; postharvest technology; and processions and marketing.

Further information is available at the conference website:

http://www.icube-t.co.jp/isc2001/

The early registration deadline was 31 July 2001, and registration after

that date is regarded and regular registration. No on-site registration will be accepted at the conference.



Cucurbitaceae 2002

8-12 December 2002
Naples Beach Hotel & Golf Club, Naples, Florida, USA

Cucurbitaceae 2002: Enhancement and Evaluation of Cucurbit Germplasm, is scheduled for Naples, Florida, during the winter of 2002. The purpose of the conference is to bring together those involved in cucurbits so we can share information on all aspects of cucurbit research, development and production. The conference will focus on genetics and breeding for variety development. Conference sessions will be comprised of presentations organized from individual paper submissions on genetics, horticulture, plant pathology, entomology and other topics. You are invited to join us for this in-depth conference on exploring the ever-changing face of cucurbit research and development.

A Call for Papers and Posters will be issued to solicit papers for individual presentations. All presentations, both oral and poster, will be selected from submitted papers. Plenary sessions will consist of invited speakers along individual oral presentations. Each topical session will be followed by time to view poster displays pertaining to the same topic. Topics will focus on plant breeding and genetics, growth and development, production, pathobiology, entomology, physiology, and production utilization and processing, along with new technologies being used in the field.

The conference will provide numerous opportunities for participants to share up-to-date information and research, and to discuss common concerns with colleagues from around the world. This exchange of information is vital to the continued improvement and international advancement of cucurbits. So mark your calendar today and make plans to attend.

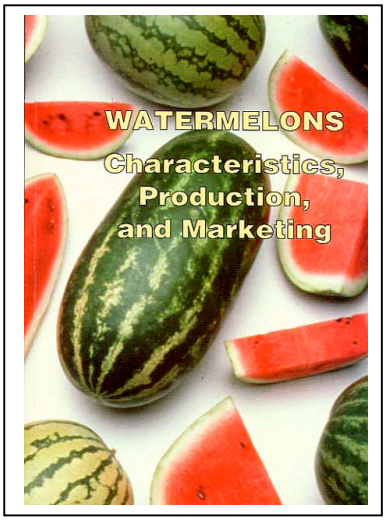
For more information, see the website at:

http://conference.ifas.ufl.edu/cucurbits

or contact the Conference Organizer: Don Maynard, University of Florida/IFAS, Gulf Coast Research and Education Center, 60th Street East, Bradenton, FL 34203. Ph: (941) 751-7636 ext 239; FAX: (941) 751-7639; Email: *dnma@mail.ifas.ufl.edu.*

New Watermelon Book

The American Society for Horticultural Science (ASHS) Press has released a new book on watermelons: "*Watermelons: Characteristics, Production and Marketing*" (D. Maynard, ed.)



Watermelon experts throughout the USA have contributed to the book, and everything from little-known facts to current pest management, production, handling and marketing practices are included.

Contents include: introduction to the watermelon; origin, distribution and uses; breeding and improvement; biotechnology; cultural management; nematode-induced maladies; diseases; insect and mite pests; weed management; harvesting and postharvest handling; marketing; and promotion and merchandising. The appendices include: watermelon organizations; watermelon seed sources; cucurbit publications; costs of production; and common names in 15 languages.

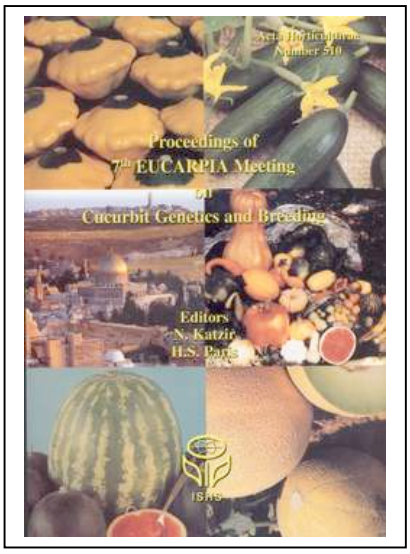
The book is $50.95 US + shipping for ASHS members, and $59.95 + shipping for non-members. You can contact ASHS press at Ph: (703) 836-4606; Fax: (703) 836-2024; or view their webpage at:

http://www.ashs.org/ashspress/watermelons.html

The book is reader-friendly, well-indexed, lavishly illustrated, and a welcome addition to anyone's library.

Conference Proceedings

Not all of us are fortunate enough to attend all of the cucurbit conferences that have occurred in the past decade. Fortunately, many of proceedings from these conferences are still available for ordering:



Proceedings of the VII Eucarpia Meeting on Cucurbit Genetics and Breeding (2000), Ma'ale Ha Hamisha, Israel. Edited by N. Katzir & H.S. Paris. Available through ISHS press at:

http://www.ishs.org/pub/510.htm

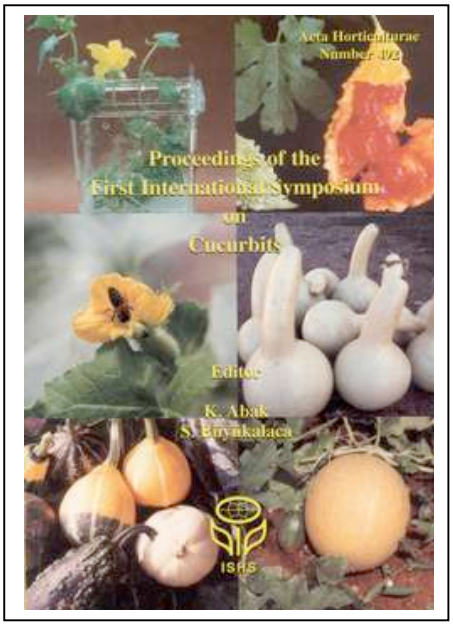
These are the proceedings from Cucurbitaceae 2000, the VII Eucarpia meeting on cucurbit genetics and breeding. The 2000 meeting was convened to present the latest developments in cucurbit genetics, breeding, germplasm enhancement, pathology and related fields. The Meeting was held at the Ma'ale Hachamisha Kibbutz Hotel and Convention Center, situated in the foot hills of the Judean Mountains in Israel.

Cucurbitaceae '98: Evaluation and Enhancement of Cucurbit Germplasm (1998). Edited by J.D. McCreight. Available from ASHS press at:

http://www.ashs.org/ashspress/cucurbit.html

These are the proceedings of the 1998 conference held at the Asilomar Conference Center in Pacific Grove, California. The conference was convened by ASHS, USDA, CGC and the Cucurbit Network.

Topics of the conference included the collection, preservation, characterization, evaluation, and enhancement of cucurbit germplasm. The six major topic areas were: germplasm resources; genetics; breeding; pathology; entomology, and production. More than 60 papers were presented at the meeting, making this volume one of the most significant sources of cucurbit germplasm currently available.



1st International Symposium on Cucurbits (1999). Edited by K Abak and S. Büyükalaca. Available from ISHS press at:

http://www.ishs.org/pub/492.htm

These are the proceedings from the ISHS symposium held in Adan, Turkey, in 20-23 May 1997. The symposium covered all scientific disciplines of relevance to cucurbits. The primary emphasis was on genetics, breeding, biotechnology, physiology, post-harvest technology, growing techniques, marketing and economics.

Other Proceedings:

CGC has a very limited supply of the proceedings from **Cucurbitaceae '94** (Padre Island, TX, USA) and **Cucurbitaceae '96** (Malaga, Spain). Please check with the CGC Chair about their availability, if interested.

The Proceedings from the **1st International Oil Pumpkin Conference** were published in CGC Report No. 24 (2000).

Upcoming Meetings

Organization/Meeting	Date(s)	Location	Contact
Cucurbit Genetics Cooperative	2:00-3:00 p.m., 22 July 2001 August 2002 September 2003	Sacramento Convention Center Sacramento, CA Toronto, Canada Providence, Rhode Island	Tim Ng 301-405-4345 *tn5@umail.umd.edu*
2nd ISHS International Symposium on Cucurbitaceae	28 Sept – 1 Oct 2001	Tsukuba City, Ibaraki, Japan	Hiroshi Ezura *ezura@gene.tsukuba.ac.jp* *http://ns.icube-t.co.jp/isc2001/*
New Crops and New Uses: Strength in Diversity	10-13 November 2001	Omni Hotel at CNN Center Atlanta, Georgia	David Dierig *ddierig@uswcl.ars.ag.gov* http://www.hort.purdue.edu/newcrop/announce/ncnu.html
Cucurbitaceae 2002	8-12 December 2002	Naples Beach Hotel & Golf Club Naples, Florida, USA	Don Maynard *bra@gnv.ifas.ufl.edu* *http://conference.ifas.ufl.edu/cucurbits/*
Eucarpia Cucurbitaceae 2004	2004	Czech Republic	Aleš Lebeda 420/68/5223325 *lebeda@risc.upol.cz*
Watermelon Research & Development Working Group	2-3 February, 2002 2003	Orlando, Florida Mobile, Alabama	Benny Bruton 580-889-7395 *bbruton-usda@lane-ag.org*
Pickling Cucumber Improvement Cooperative	24-25 October 2001	Hyatt Regency Crown Center Kansas City, Missouri	John O'Sullivan *josulliv@uoguelph.ca*
Pickle Packers International Fall Meeting Spring Meeting	24-25 October 2001 2002	Kansas City, Missouri Madison, Wisconsin	Richard Hentschel 630-584-8950 *staff@ppi.i.org*

Cucurbit Genetics Cooperative
Financial Statement
31 December 2000

Balance (31 December 1999)	**$3,398.35**
Receipts	
Dues & CGC Back Issue Orders	$2,959.00
Interest on Savings	$53.52
Total Receipts	**$3,012.52**
Expenditures	
CGC Report No. 23 (2000)	
Printing	$2,348.91
Mailing	$790.97
Call for Papers (Report No. 24)	$142.69
Member/Subscriber Renewal Notices	$80.10
Bank Fees & Adjustment Charges	$0.00
Miscellaneous (envelopes, postage, etc.)	$37.33
Total Expenditures	**$3,400.00**
Balance (31 December 2000)	**$3,010.87**

Determination of the Crossing Barriers in Hybridization of *Cucumis sativus* and *Cucumis melo*

V. Ondřej, B. Navrátilová and A. Lebeda
Palacký University, Faculty of Science, Department of Botany, Šlechtitelů 11, 783 71, Olomouc, Czech Republic
Email: *Ondrej.Vladan@seznam.cz, lebeda@prfholnt.upol.cz*

Introduction: Serious crossing barriers prevent the successful hybridization of *Cucumis sativus* L. and *Cucumis melo* L. (5,6). However, such hybridization would be important for transferring several resistances from *C. melo* or other wild *Cucumis* spp. to *C. sativus* (1,4,5). The determination of crossing barriers can help in selecting potentionally successful methods for overcoming obstacles to fertilization. For example, *in vitro* pollination followed by *in vitro* cultivation of rescued hybrid embryos without challenge to extirpation shocks *in ovulo* (9, 13) could be used as a methodology.

Interspecific crossing barriers can be classified into two groups: (a) prezygotic (including all factors hindering effective fertilization), and; (b) postzygotic (occurring during or after syngamy) (14). Experiments were designed to overcome these barriers in *C. sativus* x *C. melo* mating by *in situ* and *in vitro* pollination. The first stages of embryos development were observed to investigate the responses to treatments for overcoming fertility barriers.

Material and Methods: Plants of *Cucumis sativus* (line SM 6514) and *Cucumis melo* (cv. Solartur) were grown in a glasshouse. The seeds originated from the Vegetable Germplasm Collection of the Research Institute of Crop Production, Prague, Gene Bank Division, Workplace Olomouc, Czech Republic. Female flowers at the stage of anthesis were self-pollinated or pollinated with pollen of the opposite *Cucumis* species.

The observation of *in situ* fertilization was made by cutting of pollinated pistils and staining in aniline blue. The stained slides were observed by fluorescence microscopy. The observations of pistils were made 6, 24 and 48 hours after hand-pollination.

The pollen grains and ovules for *in vitro* observation were aseptically isolated onto a YS culture medium (10) (Table 1) for use in fertilization experiments. The process of *in vitro* fertilization was observed via inverted microscopy. After 20 hours the ovules were transferred onto DI1a culture medium (Table 1) (2) in Petri dishes. They were cultivated in a growth chamber under light intensity 32 to 36 μmol (PAR) $m^{-2}s^{-1}$, with light/dark cycles being 16/8 hrs and temperature 22 $\pm$ 2 °C.

Seven days after *in situ* and *in vitro* pollination, the immature seeds were taken for embryological analyses. Seeds were fixed in Carnoy solution, cutted in paraffin and stained in hematoxyline.

Results and Discussion: *Differences in fertilization of C. sativus, C. melo and interspecific hybrids.* Cucumber and melon ovules were of the anatropous type and contained a monosporic, Polygonum-type embryo sac (3). The pollen grains of both species were triporate and contained vegetative and generative nuclei during cell maturation. The size of *C. sativus* grains was about 60 μm, and *C. melo* was about 50 μm (11).

Twenty to 30 minutes after *C. sativus* self-pollination, pollen grains began to germinate on the stigma. Six hours after pollination, pollen tubes were observed on the stigma-style border. Twenty-four hours after pollination the pollen tubes were localized on the style – ovary border. The pollination process in *C. melo* was slower than in *C. sativus.* During the first 6 hours the pollen tubes were still in stigma, and at 24 hours in style. The penetration of ovules and fertilization occurred 48 hours after pollination.

During hybridization of *C. sativus* (male) x *C. melo* (female) and *C. melo* (male) x *C. sativus* (female), abnormalities were not found in pollen tubes, and during their germination and development. Likewise, no comparative differences in the growth and speed of tube maturation were observed after self-pollination of parental stocks.

Table 1. The composition of culture medium used *in vitro* pollination and ovule cultivation of interspecific hybrid progeny between *C. melo* and *C. sativus*.

Composition	Type of medium	
	DI1a	YS
Macro and micronutrients (mg/l)	MS	600 $Ca(NO_3)_2xH_2O$ & 100 H_3BO_3
Vitamins (mg/ml)	MS & 5 vit. PP	none
Amino acids (mg/ml)	MS	none
Protein hydrolysates (g/l)	0,4 CH	none
Sucrose (g/l)	30	80
Agar (g/l)	8	10
Growth regulators (mg/l)	4 IAA	none
	2,5 Kin	
	0,4 2,4-D	

MS - Murashige and Skoog medium (7)

CH - casein hydrolysate

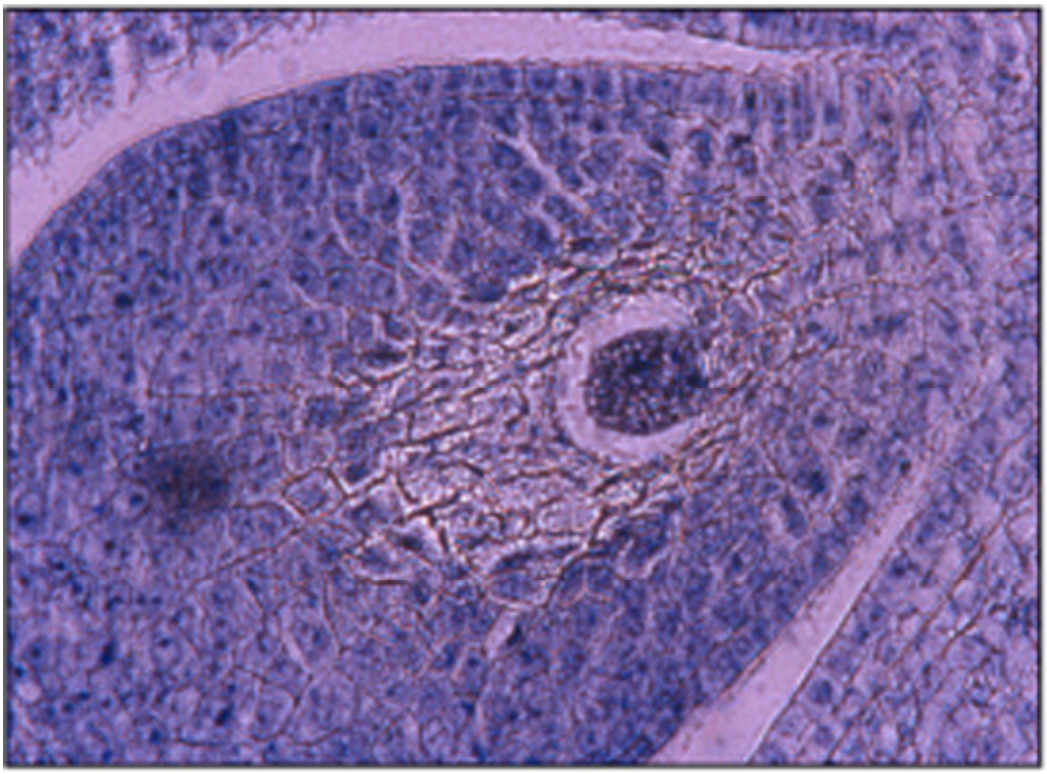


Figure 1. Globular embryo of *C. sativus* seven days after *in situ* pollination.

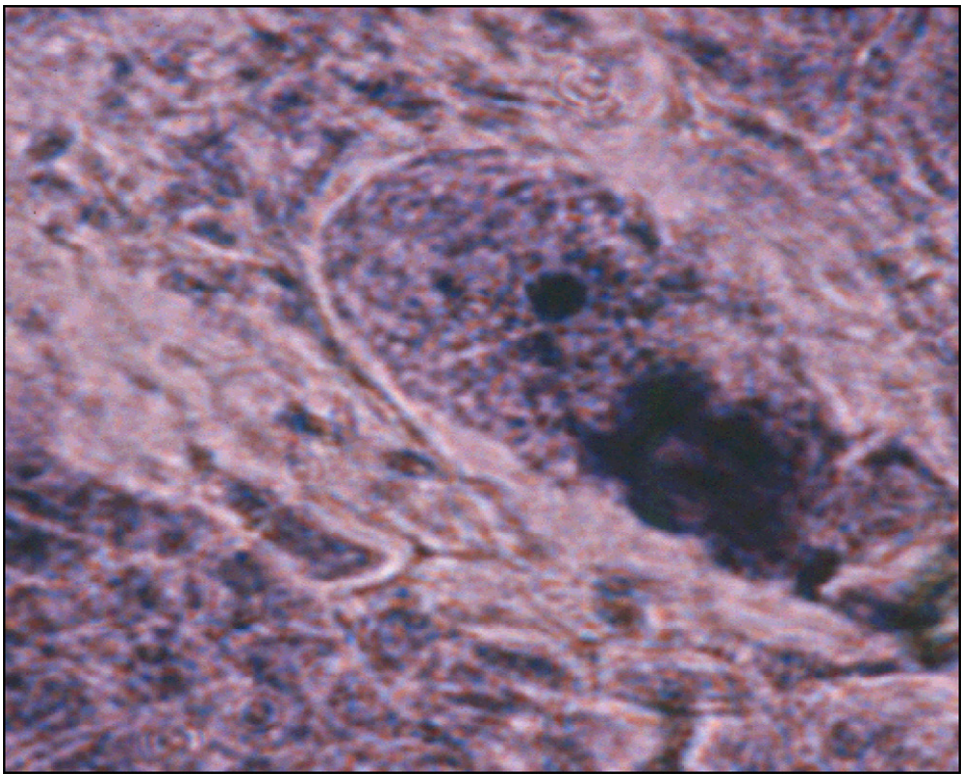


Figure 2. Hybrid embryo of *C. sativus x C. melo* seven days after *in situ* pollination.

Prezygotic barriers have previously been found during interspecific crosses *C. melo* x *C. metuliferus* (1), or by crosses of *C. melo* (2n) x *C. melo* (4n) (10). However, in our experiments, these kinds of barriers were not observed. Seven days after self-pollination of *C. sativus* (Fig. 1) and *C. melo* plants, globular embryos developed. Thereafter, normal development of embryos, seeds and fruits was recorded.

The hybrid immature seeds contained globular embryos seven days after pollination (Fig. 2). However, the development of embryos and fruits stopped at this stage. Embryos aborted and immature fruits became yellow in color. This developmental sequence is in agreement with previously published data (8). The abortion of hybrid embryos (postzygotic barrier) could be considered as a main factor in the inability of these *Cucumis* species to cross fertilize.

Differences in fertilization in situ and in vitro. The pollen germination *in vitro* started 10 min after transfer to the culture medium. This was a shorter time than that demonstrated by germination *in situ*. The suitability of medium for germination *in vitro* was demonstrated by the absence of cracked pollen tubes and calloses in tubes. The highest concentration of boric acid and sucrose stimulated pollen germination and pollen tube growth. The tubes length was around 450 μm one hour after germination in both species, and 1350 μm for *C. sativus* and 1100 μm for *C. melo* 24 hours after cultivation. At that time, the tubes growth stopped. In contrast to pollination *in situ*, no taxis of tubes were observed; except for a very small area near the ovules.

Penetration of ovules by pollent tubes was noted and globular embryos were detected in both species and their hybrid seven days after cultivation. These results showed that the complete process of sexual reproduction can be accomplished in *C. sativus* x *C. melon* matings, and that embryos can be obtained for *in vitro* cultivation at an early stage of development after fertilization.

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Cucumber Inbred Line USDA 6632E

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Release Announcement: Line 6632E, a multiple-disease resistant, gynoecious, processing cucumber (*Cucumis sativus* L.) inbred is being released by the U. S. Department of Agriculture Agricultural Research Service to provide breeders with a line having high fruit quality and multiple disease resistance to produce hybrid cultivars and germplasm for breeding purposes. The disease resistance attributes of USDA 6632E originates from previously released USDA inbred lines WI 1379 (4), 'Wautoma' (2), and WI 2870 (3). The resistance identified in greenhouse evaluation for anthracnose and angular leaf spot has been confirmed in field tests at the University of Wisconsin Experiment Station, Hancock, WI (HES).

Origin: USDA 6623E originated from a cross made in 1987 between the multiple disease resistant USDA line 3733 and the monoecious multiple disease resistant line, 'Wautoma'. The F_1 hybrid was self-pollinated to produce F_2 progeny that were selected at HES in 1988 for gynoecious and high fruit quality characteristics (e.g., fruit length and small seed cavity). Rooted cuttings of selected plants were crossed to the gynoecious, multiple disease resistant USDA line 2870. Subsequently, the progeny from this cross were selected for gynoecious and high fruit quality at HES in 1990. Selections were subjected to continued selection for these characteristics and self-pollinated to produce F_5 inbred lines and then increased under cage isolation to produce seed for replicated trials (Table 1).

Description of inbred lines used to create USDA 6632E: Line 3733 [(USDA line 1983 x Gy-2)F_6] is gynoecious, indeterminate, and produces non-bitter, white-spined fruit of a length:diameter ratio (L:D) equal to 3.0 (not released). The gynoecious, multiple disease resistant USDA line 1983 was released in 1983 (1). Line 3733 is resistant to angular leaf spot [*Pseudomonas lachrymans* (E.F. Smith and Bryan) Carsner; ALS], anthracnose [*Colletotrichum orbiculare* (Berk. & Mont.) Arx; AN], cucumber mosaic virus (CMV), *downey mildew* [Pseudoperonospora cubensis (Berk. & Curt.) Rostow; DM], *powdery mildew* [*Sphearotheca fuliginea* (Schlechet. Ex Fr.) Poll.; PM], and Scab (spot rot) (*Cladosporium cucumerinum* Ellis & Arthur; SC). Line 1379 {[(3121 x Gy14) x $Gy14^2$]S_4} is indeterminate, resistant to SC, CMV, PM, DM, ALS, AN and produces non-bitter, white-spined fruit of L:D 3.0. The indeterminate, disease resistant (SC, CMV, PM, AL, AN, DM) line 2870 produces white-pined, non-bitter fruit of L:D 3.0. 'Wautoma' is resistant to eight destructive diseases including SC, CMV, ALS, DM, PM, AN, fusarium wilt [*Fusarium oxysporum* (Schlect.) Synd. & Hans.] and TLS [*Corynespora cassiicola* (Berk. & Curt.)], and produces non-bitter, white-spined fruit of L:D 3.1.

Description of USDA 6632E: Line 6632E is indeterminate, gynoecious and resistant to ALS, AN, DM, CMV, PM, and SC. It is has field resistance to TLS. Fruit average about 3:0 in L:D (Table 1); are bitter free, white-spined, typically moderate to dark green depending on growing environment, and nearly cylindrical. Line 6632E flowers about the same time as 'Vlasset'. In our 1997 to 2000 trials, line 6632E preformed as well as standard monoecious hybrids evaluated for fruit yield and brining quality. Line 6632E has been tested in hybrid combination with lines 6812A and 6849A to produce hybrids that are competitive with the fruit yield and quality of 'Vlasset' and other standard commercial cultivars (data not shown).

Availability: Breeder's seed, produced under screen isolation, will be provided to U.S. hydrid-seed producers and cucumber breeders by J.E. Staub, ARS/USDA, Dept. of Horticulture, Univ. of Wisconsin, Madison, WI 53706.

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Table 1.Comparative means and LSDs over evaluation seasons for USDA line 6632E and various experimental (M21 and 2870) and a commercial cucumber hybrids.

		Days to	Avg of 4 Harvests[z]			Overall	Firmness[v]	
Trial year	Trial entry	flower	No.	Wt.[y]	L/D	brine rating[w]	Blossom	Stem
2000	6632E A X M21	39.8	9.6	2.0	3.0	2.4	19.1	20.5
	6632 E	39.8	9.6	1.6	2.8	2.8	19.5	20.0
	Vlasset	40.2	9.6	1.8	2.9	3.5	20.9	22.3
	LSD (0.05)	3.1	1.8	0.4	0.1	0.4	1.7	2.2
1999	6632E X M21	38.5	8.0	1.7	3.0	2.8	17.2	18.0
	6632E X 2870	38.8	8.0	1.8	2.8	3.1	19.4	19.2
	6632E	38.8	7.8	1.4	2.9	3.4	19.3	20.6
	Vlasset	39.2	8.1	1.6	2.7	3.1	20.2	21.8
	LSD (0.05)	1.6	1.4	0.4	0.1	0.4	2.4	2.6
1998	6632E X M21	39.3	10.8	2.0	3.1	2.2	14. 8	14.8
	6632E X 2870	38.8	8.7	2.1	2.8	2.9	16.6	16.7
	6632E	39.2	7.6	1.1	2.9	1.7	13.8	14.0
	Vlasset	39.7	8.8	1.6	2.8	2.7	17.3	18.2
	LSD (0.05)	1.5	1.9	0.7	0.3	0.4	2.0	2.4
1997	6632E X M21	42.0	6.5	1.4	3.2	3.7	14.4	17.2
	6632E X 2870	42.8	6.5	1.4	3.0	2.9	15.1	17.2
	6632E	40.5	7.1	1.2	3.0	3.3	11.8	15.3
	Vlasset	39.0	7.5	1.4	2.8	3.7	15.7	17.1
	LSD (0.05)	1.5	1.8	0.5	0.2	0.4	2.0	2.5

[z] Fruit number and weight presented on a per plant basis where plants were set at approximately 20,000 plants per acre. L/D = length:diameter ratio.

[y]Weight in pounds.

[w]Data are means over 12 judges and 3 replications. Fruit scored 1 to 5, where 1 = excellent 3 = moderate, and 5 = unacceptable.

[v]Punch test using Magness-Taylor pressure tester, with 7.9 mm tip. Average for ten, 38-mm diameter fruits, taken from each replication at 3rd harvest.

Multiple Shoot Induction from the Shoot Tip Explants of Cucumber *(Cucumis sativus L.)*

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Introduction: Cucumber is an important horticultural crop in India, cultivated primarily for its fruits for slicing and pickling, juice extraction, and for the preparation of traditional Indian medicines. However, the crop is known to be susceptible to abiotic as well as biotic stress (5). Applications of conventional techniques for its genetic improvement are limited because of interspecific incompatibility. A tissue culture method of propagation that could produce numerous plants would be desirable to produce a sufficient number of plants for inbreeding (2). A system suitable for transformation should ideally produce multiple shoots with minimal peripheral callus production so that the transformation of many of these individual buds is possible (1). This paper describes a technique which allows the production of multiple shoots from adventitious buds in cucumber shoot tip explants.

Materials and methods: Seeds of 'Poinsett 76' cucumber (Petoseed Co., Inc., USA) were soaked in tap water for 15 min. The seeds were surface sterilized with 70% alcohol for 1 min and then 25 % (v/v) commercial bleach " Teepol " (5.25% sodium hypochlorite) (Reckit & Colman of India Ltd., India) for 15 min. The seeds were then thoroughly washed three times with sterile distilled water and kept in 0.1% mercuric chloride solution (w/v) for 10 min. Finally the seeds were rinsed four times in sterile distilled water to remove the sterilant. The seeds were germinated in darkness for 48 hrs on sterile moist cotton.

Shoot tips (5mm length) from 5-7 day old in vitro grown seedlings were excised and used as explants. The explants were inoculated on MS medium (3) containing 3% sucrose (Himedia Co., Mumbai, India) with different auxin (2,4-D, NAA, and IAA) and cytokinin (BAP and Kin) compositions and concentrations. The pH of all the media was adjusted to 5.8 before autoclaving at 121°C for 20 min. The cultures were kept at 25±2°C with a 16 h photoperiod under diffused cool-white fluorescent lamps (80 µ mol $m^{-2}.s^{-1}$). Individual shoots were carefully excised from the shoot clusters arising from the explants and subcultured on media containing the same hormone composition.

Results and Discussion: *Effect of cytokinins on multiple shoot induction:* Shoot tips from 5-7 day old seedlings were used as explants. Adventitious buds were induced from the meristematic regions of shoot tip explants planted on media containing BAP (0.5 – 1.0 mg/l) or Kin (1.0-2.0 mg/l) after 10 days of inoculation. A maximum number of shoots was obtained from adventitious buds after 4 weeks of culture on the medium supplemented with 1.0 mg/l BAP. Lower concentrations of BAP and Kin (below 0.5 mg/l), however, did not promote bud/shoot induction. Between these two hormones, the effects of BAP was greater in producing a higher number of shoots (Table.1). Individual shoots were isolated from the shoot clusters and subcultured in the medium containing the same composition.

Effects of combinations of cytokinins with auxins: Shoot tips of 7 day old seedlings were used as explants. Adventitious buds were initiated from the meristematic regions of the shoot tip explants after 2 weeks inoculation in the treatments containing BAP and NAA at concentrations of 1.0 mg/l and .2 mg/l respectively. In other concentrations of BAP and NAA calluses were produced preventing shoot bud induction. At all concentrations and combinations of 2,4-D/BAP and IAA/BAP, only calluses and subsequent rhizogenesis were noticed (data not shown).

The explants tended to produce callus on media supplemented with BAP and 2,4-D or NAA or IAA at higher concentrations. As many as 22 plant were produced from a single shoot tip explant planted on media containing BAP (1.0 mg/l) and NAA (0.2 mg/l) in successive subcultures in the medium with the same composition. The present study revealed that individual treatments of either BAP or kinetin produced shoots from shoot tip explants, but the number of shoots per explant was low. A low

Table 1. Effect of BAP on multiple shoot induction from the shoot tip explants of *Cucumis sativus*

MS medium + PGR (mg/l)	Number of shoots/explant	Shoot length (cm)
BAP		
0.2	n.d	n.d
0.4	1.0 c	4.8 cd
0.6	3.0 bc	5.2 c
0.8	5.0 b	5.9 ab
1.0	8.0 a	6.1 a

n.d: not determined due to nil response.
The experiment was repeated three time with 20 replicates.
Means followed by same letters within a column are not significantly using Duncan's Multiple Range Test (DMRT)

Table 2. Effect of kinetin on multiple shoot induction from the shoot tip explants of *Cucumis sativus*

MS medium + PGR (mg/l)	Number of shoots/explant	Shoot length (cm)
Kinetin		
0.2	n.d	n.d
0.4	n.d	n.d
0.6	1.0 bc	4.1 b
0.8	2.0 b	4.3 ab
1.0	4.0 a	4.9 a

n.d: not determined due to nil response.
The experiment was repeated three time with 20 replicates.
Means followed by same letters within a column are not significantly using Duncan's Multiple Range Test (DMRT)

Table 3. Effects of combinations of BAP and kinetin with NAA on multiple shoot induction.

MS medium + PGR (mg/l)		Number of shoots/explant	Shoot length (cm)
BAP	NAA		
1.0	0.1	16.0 b	2.2 ef
	0.2	22 a	8.3 a
	0.3	11 c	7.0 b
	0.4	n.d	n.d
	0.5	n.d	n.d
Kin	NAA		
1.0	0.1	5.0 de	1.1 f
	0.2	9.0 cd	2.3 c
	0.3	6.0 d	4.1 d
	0.4	2.0 e	5.4 c
	0.5	n.d	n.d

n.d: not determined due to nil response.
The experiment was repeated three time with 20 replicates.
Means followed by same letters within a column are not significantly using Duncan's Multiple Range Test (DMRT)

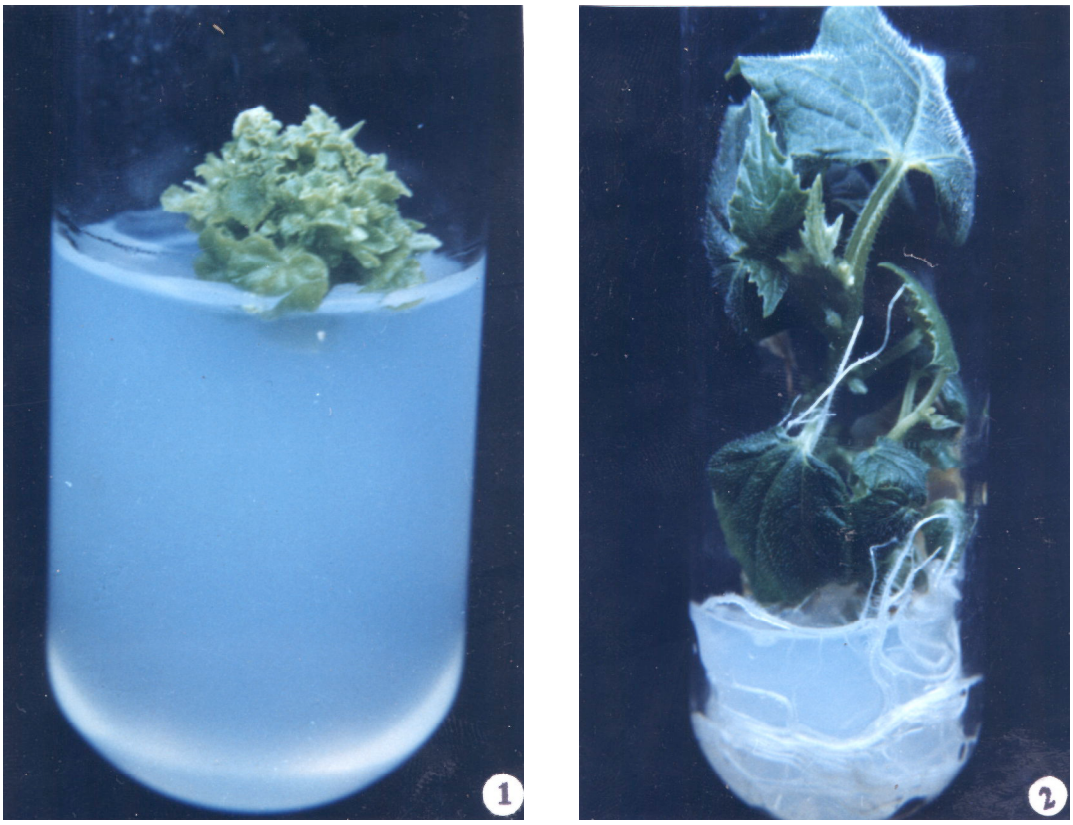

Figure 1. Emergence of multiple shoots (shoot cluster) from the shoot tip explants of 7 day old seedlings of 'Poinsett 76' cucumber.

Figure 2. Elongated shoot excised from the shoot cluster.

concentration of NAA (0.1 mg/l) with optimal concentrations of BAP (1.0 mg/l) was found essential in the induction of adventitious buds and subsequent multiple shoots from the shoot tip explants. At least three subcultures were required to produce the maximum number of shoots per explant. No phenotypic changes were observed in control plants and plants derived from the shoot tip cultures. The system described would be applied for transgenic recovery and in breeding programs. Our results are in agreement with the observation of Plader et al. (4).

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Stimulatory Effects of Different Cytokinin on Direct Plant Regeneration from Cotyledon Explants in *Cucumis sativus* L.

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In order to establish a regeneration system for gene transformation, the effect of four kinds of cytokinins (KT, BA, ZT and TDZ) were studied on direct shoot regeneration from cotyledons of 5-day-old seedlings of *Cucumis sativus* L. inbred line Jin431. ZT had a highest efficiency among the four cytokinins, and its regeneration frequency of 85% with multiple shoots may be useful for gene transformation. TDZ had the highest activation, and the optimal concentration of TDZ was considerably lower than that of the other three cytokinins. A new phenomenon, in which roots were induced as well as shoots by TDZ, was found.

Keywords: cucumber; cytokinin; organogenesis; cotyledon

Introduction: Cucumber (*Cucumis sativus* L.) is an important species of Cucurbitaceae and, with the exception of watermelon, is cultivated more than any other cucurbit in China. Recent attention has been directed towards gene transformation technology as a way of introducing heterogeneous genes into cucumber without the limits of cross incompatibility between species. There have been about 14 studies on cucumber gene transformation by the way of *Agrobacterium*-medium or microprojectile bombardment from 1986. Only a few of these studies have obtained transformants. Gene transformation systems depend on some form of plant regeneration technology. Most of the studies dealt with indirect plant regeneration. A high frequency of variation in regenerated plants occurs at the callus or somatic embryo stage (Malepszy & Nadolska-Orczyk, 1989). Some studies found that direct shoot regeneration (organogenesis) from explants in cucumber were free of morphological or physiological variation (Burza & Malepszy, 1995; Plader et al., 1998). A system suitable for transformation should ideally produce multiple shoots from numerous adventitious buds with minimal callus. This paper describes the effects of different cytokinins on organogenesis.

Materials and Methods: *Explant materials.* Seeds of *Cucumis sativus* L. inbred line Jin431 were surface-sterilized for 10 min using a dilution of commercial sodium hypochlorite having 3.5% active chlorine, then rinsed three times in sterile deionized water. The seeds were germinated on modified Murashige & Skoog (MS, 1962) basal medium supplemented with 3% sucrose and solidified with 0.75% agar. The pH was adjusted to 5.8 with 0.1N NaOH prior to sterilization at 121 C for 15 min. All plant material was incubated at 25 C with 16 hr photoperiod at 5.0 klx provided by cool white fluorescent lighting. Cotyledons from five-day-old seedlings were excised and cut transversely, and the basal segments without axillary buds were used for all experiments. Explants were placed with the abaxial side down on shoot induction media with the basal end just beneath the surface of the medium.

Methods: In the preliminary experiments, it was found that cytokinin was sufficient for shoot induction without auxins, while auxins can increase callus growth and decrease shoot development. In order to investigate the influence of different cytokinins on shoot induction and to select the optimal concentration, shoot induction media were prepared by modifying the MS medium above with the following concentrations of individual cytokinins: (1) kinetin (KT) concentrations at 0, 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 mg l^{-1}, (2) 6-benzylaminopurine (BA) concentrations at: 0, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 mg l^{-1}, (3) zeatin (ZT) concentrations at: 0, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 mg l^{-1}, (4) and thidiazuron (N-pheny1-N'-1,2,3-thiadiazol-5yl-urea, TDZ) concentrations at: 0, 0.0001, 0.001, 0.005, 0.01, 0.05, and 0.1 mg l^{-1}. (KT, BA and ZT were obtained from Sigma Co., while TDZ was synthesized by the Northwest Plant Institute, Chinese Academy of Sciences.)

Explants were cultured on shoot induction media under the same conditions as seedlings. There were 10 replicates per treatment with four explants per

replicate. Shoot differentiation and induction frequencies were investigated at the 20th day after induction. Statistical analysis was conducted according to a single-factor design.

Results: Protuberances could be found at the basal region of cotyledons after 7-8 days induction when cotykinin concentrations were suitable, and little buds could be found after two weeks. These buds could develop to 1-2 cm in size after 3 weeks (Fig.1).

The effects of a variety of different cotyledons on shoot induction, and statistical analyses of KT, BA and ZT concentrations are shown in Table 1, while the results of shoot induction frequencies and statistical analysis of TDZ are shown in Table 2, since the range of TDZ concentrations was much lower that the other three. The results of the four kinds of cytokinins exhibited the same tendency in that shoot induction frequency was low and the callus grew slowly in media with low cytokinin concentrations, shoot induction became optimal and the callus grew moderately with increasing cytokinin concentrations, and shoot induction then decreased sharply and while callus growth was greatest with the further increases in cytokinin concentration, although the callus grew little at the highest concentration of cytokinin. The cytokinins KT, BA and ZT could induce multiple shoots at the basal region of cotyledons in the media with each cytokinin's optimal concentration. The number of shoots induced per explant was usually more than 10, and 25-30 at maximum. The cytokinin TDZ induced fewer shoots at the base of the cotyledon than the other three cytokinins, with fewer than 3 shoot developed per explant. There was a new phenomenon in that roots were induced as well as shoots in the shoot induction medium with a TDZ concentration of 0.0001 to 0.01 mg l^{-1} (Fig. 2). The root induction frequency increased with decreasing concentration.

The optimal concentration of KT was 5.0 mg l^{-1}, of BA was 0.5 mg l^{-1}, of ZT was 1.0 mg l^{-1}, and of TDZ was 0.005 mg l^{-1}. The cytokinin TDZ had a higher activation on shoot induction than the other three, and the optimal concentration of TDZ was considerably lower. ZT had the highest efficiency (85%) of shoot induction of the selected inbred line. The concentration range of cytokinins on shoot induction differed from each other. TDZ could induce shoots at concentration of 0.00001 to 0.05 mg l^{-1} and ZT had a relative high effective at concentration of 0.2 to 5.0 mg l^{-1}, while KT and BA had a relative narrow range of concentration on shoot induction.

Discussion: Wehner and Locy (1981) studied the direct regeneration with cotyledon and hypocotyl from 7-day-old seedlings as explants on the MS medium containing 1.0mgl BA and 1.0 mgl^{-1} NAA. Eighty-five cultivars were tested and just 28 cultivars developed shoots with an average shoot induction frequency of 5.5%. That is likely due to the addition of NAA and explants from elder seedling. In order to establish the cucumber gene transformation system, Gambley and Dodd (1990,1991) studied successfully the shoot direct regeneration with three kinds of cytokinins KT, BA and 2-ip (N^6-(2-isopentenyl)adenine). They found that concentration (4 mgl^{-1} or less) of the cytokinins KT, BA and 2-ip with or without low concentration of auxin, were all effective in producing multiple adventitious buds at the base of cotyledons from 5-7 -day-old seedling. BA had a best efficiency in the three kinds of cytokinins. In this studied, the similar results were obtained. We found that the auxin restrained the shoot development even at low concentration in the preliminary experiments (data not showed), and the ZT had a highest efficiency on inducing shoots of the selected inbred line among the four kinds of cytokinins. So this multiple adventitious shoots regeneration technique should be useful for transformation studies in cucumber. We had got some of chitinase/ ß-1, 3-glucuronidase genes and glucose oxidase gene transformants by this direct organogenesis technique.

On the other hand, we observed a new phenomenon that roots were induced as well as shoots were induced. That may be due to the higher endogenesis auxins compared with the absolute amount of TDZ.

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Table 1. Shoot induction efficiency of three kinds of cytokinins

cytokinins	Concentration (mg/l)							
	0	0.2	0.5	1.0	2.0	5.0	10.0	20.0
KT	0dC		5cdBC	20bB	20bB	50aA	15bcB	0dC
BA	0dC	20cC	79aA	70abAB	55bB	8dC	0dC	
ZT	0cC	45bB	60bAB	85aA	50bB	39bB	0cC	

Notes: a,b,c and d indicate significance at the 0.05 level.
A, B and C indicate significance at the 0.01 level

Table 2. Shoot induction efficiency of different concentration of TDZ

Concentration (mg/l)	Shoot induction (%)	significance level (0.05)	significance level (0.01)
0.005	60	a	A
0.01	35	b	B
0.05	25	bc	BC
0.001	20	c	BC
0.0001	5	d	CD
0.1	0	d	D
0	0	d	D

Notes: a,b,c and d indicate significance at the 0.05 level.
A, B and C indicate significance at the 0.01 level

Fig. 1 Shoots induced by cytokinin ZT.

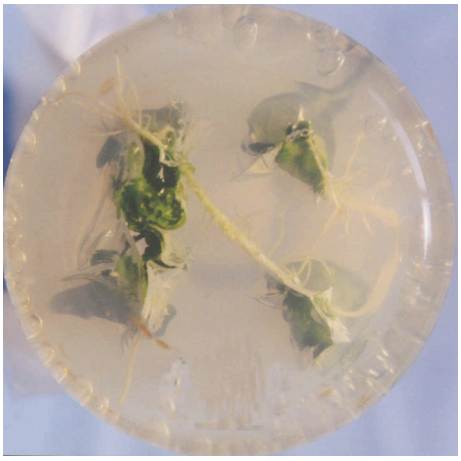

Fig. 2 Shoot and roots induced by cytokinin TDZ.

Mesophyll Protoplasts of Some Wild and Cultivated *Cucumis* spp.

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Introduction: Isolation and somatic fusion of protoplasts may provide a means of genetic interchange with the aim to create interspecific or intergeneric hybrids between *Cucurbitaceae* (1,3). Recently there are available only a few results related to this topic (4,5,6,7,8). The aim of this work was to select suitable accessions of *Cucumis* spp. for protoplast isolation, culture and fusion.

Material and Methods: Seeds of *Cucumis anguria* ssp. *longipes* (09-H41-00569), *Cucumis melo* L. (line MR-1), *Cucumis metuliferus* E. Meyer ex Naudin (H41-0587), *C. sativus* (SM-6514) and *C. zeyheri* (09-H41-0196) originated from the Vegetable Germplasm Collection of the Research Institute of Crop Production (Prague), Gene Bank Division, Workplace Olomouc, Czech Republic.

Seeds of all accessions were decoated, surface sterilized for 10 min in 2,5 % Chloramine B (sodiumbenzensulfo-chloramidium), 3-5 times rinsed in sterile distilled water. Sterilized seeds were germinated onto OK medium: mineral salts and vitamins of basic M-S supplemented with 20 g sucrose, 0,01 $mg.l^{-1}$ IBA, 0,01 $mg.l^{-1}$ BAP and 20 $mg.l^{-1}$ ascorbic acid in glass tubes (in diameter 17 mm). After one or two weeks the seedlings were transferred onto fresh OK medium in Erlenmayer flasks (100 cm^3). Cultivation took place at light/dark cycles 16/8 hrs (irradiance of cca 35 $\mu mol\ m^{-2}\ s^{-1}$) and temperature of 22 $\pm$ 2 °C. The plantlets were micropropagated to obtain clones (originated from one zygotic embryo).

The protoplasts were isolated from fully developed leaves (first and second) of plantlets cultivated *in vitro*, with good growing and rooting. Fresh leaves (about 100 mg) were cut into narrow strips, placed into 2 ml of enzyme solution consisting of 1% cellulase Onozuka R 10 (SERVA, Heidelberg) and 0,25% macerozyme R 10 (SERVA, Heidelberg) in wash medium PGly (2), then incubated 18 h in the dark at 25 °C (overnight). The crude protoplasts suspension was purified by filtration (polyamide net, 54 µm) and centrifugated at 100 G for 5 min, the pellet was resuspended in 4 ml of 20 % sucrose and overlayed by 2 ml of wash medium (PGly), centrifugated at 100 G for 5 min. Floated protoplasts (ring) were collected, resuspended in a wash medium (PGly)), centrifugated and the final pellet was resuspended in a liquid cultivation medium LCM1 (2) at the density $1\text{-}2.10^5$ $protoplasts.ml^{-1}$ (by haemocytometr). The viability was determined by FDA staining after isolation.

The protoplasts were cultured for 2 weeks in the dark at 25 °C in Petri dishes (35 mm). After this period 1 ml of liquid media LCM2 (2) was added to each dish and cultivation continued in the light/dark 16/8 hrs cycles at 22 $\pm$ 2 °C.

Results and Discussion: Plant regeneration from protoplast culture has been reported for some important genotypes of *Cucumis* spp., mainly on *C. melo* (2) and *C. sativus* (8).

In our experiments there were used five *Cucumis* species for protoplast isolation. There were used mesophyll protoplasts for obtaining the homogenous material. The protoplasts were isolated from *in vitro* plants. Growing of *C. anguria*, *C. melo, C. metuliferus* and *C. zeyheri* plantlets was very good. The plants were green with efficient rooting and micropropagation. However, the growth of *C. sativus* genotype was questionable because the plantlets were small and soon flowering, the propagation *in vitro* was rather difficult.

The success of protoplast isolation was evaluated on the basis of the density and the viability immediately after isolation. *C. metuliferus* had the highest density in all experiments (7), *C. anguria* had the highest viability. The viability of other genotypes was high and suitable for protoplast cultivation, as well as the density. The Figs. 1 and 2 show the differences between studied genotypes.

Figure 1. The viability of *Cucumis* spp.

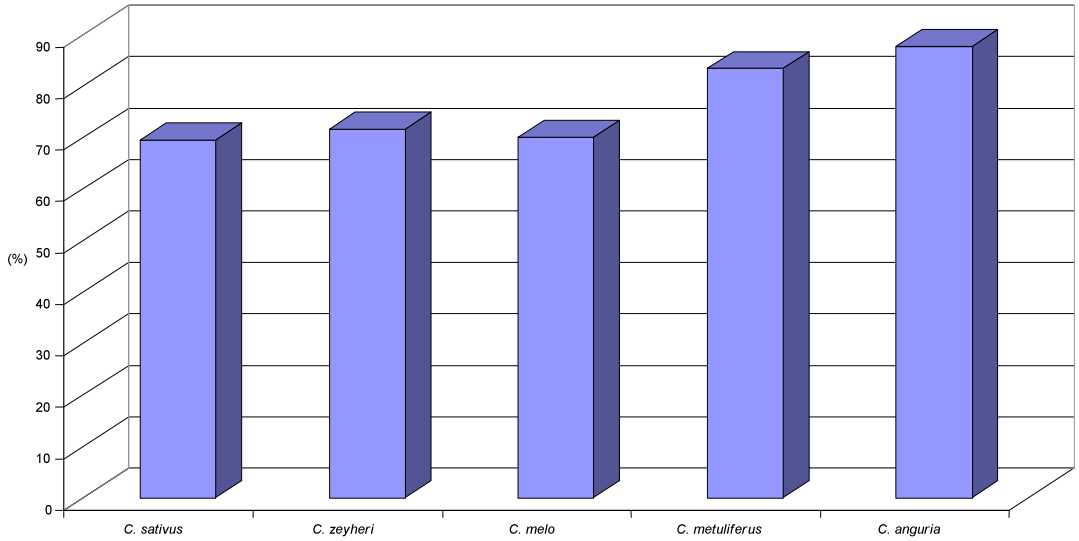
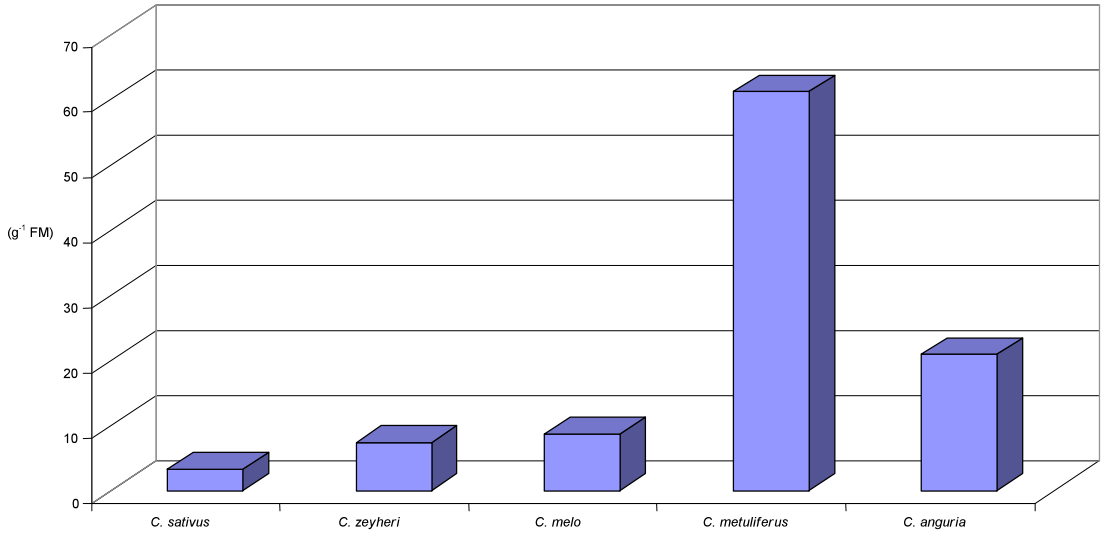


Figure 2. The density of *Cucumis* spp.



The protoplasts of *C. sativus* and *C. zeyheri* were more damaged and plasmolysed after isolation in comparison with *C. anguria*, *C. metuliferus* and *C. melo*. The viability of protoplasts decreased on 50 % during 24 hrs of culture. Changes in the form and fragmentation of all studied genotypes were recorded in the culture period 24-48 hrs.

The first division in culture was observed from seven to ten days after isolation and viable protoplasts after two weeks. Then the division was stopped and there were no obtained microcalli, calli and plantlets. Protoplast culture of *C. anguria* was without the division, although the highest viability and good density of protoplast.

In general, the best genotype (from the studied set) for protoplast isolation was *C. metuliferus* because of its easy mipropropagation, high density and viability of protoplasts after isolation. *C. melo, C. zeyheri* and *C. anguria* can be used too, but the optimalisation of culture conditions is necessary. Finally, for successfull regeneration of plants from protoplasts there is the most important to find the best genotype.

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Gynogenesis in a Dihaploid Line of Cucumber (*Cucumis sativus* L.)

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Introduction. Until now, haploid cucumber plants (n = x = 7) have only been obtained by pollinating with irradiated pollen (e.g., gamma rays from Co^{60}), followed by haploid embryo culture (Truong-Andre, 1988; Niemirowicz-Szczytt and Duma de Vaulx, 1989; Sauton, 1989; Przyborowski and Niemirowicz-Szczytt, 1994). Attempts to obtain cucumber haploids by different methods, including anther culture, have not been successful.

Haploid plants cannot be used directly in heterosis breeding programs because of their complete sterility. Doubling the haploid chromosome number offers the possibility of creating fertile dihaploid (DH) genotypes and stabilizing homozygous DH lines (Nikolova and Niemirowicz-Szczytt, 1995; Çaglar and Abak, 1996). The objective of this study was to evaluate the usefulness of some DH lines in a cucumber genetics and breeding program, and to establish characteristics of their development and plant and fruit morphology.

Material and Methods. For this study, the dihaploid (DH) line No. 50 (gynoecious type, pickling cucumber tolerant to powdery mildew, downy mildew and CMV) and the diploid line K2 (monoecious type, pickling cucumber tolerant to CMV) were used. For creation of F_1 plants, DH line No. 50 plants were pollinated with K2 pollen during 1999. The resulting seeds were germinated in perlite in a greenhouse, and seedlings at the two-leaf stage were transferred to 15 cm pots. The F_1 plants were cultivated under greenhouse conditions during April-August 2000. Their development, plant sex and shape, leaf dentation, flower petal shape, fruit morphology, and marketable and total fruit yield were evaluated.

Results and Discussion. During 2000, a variety trial was conducted which included the F_1 generation (line No. 50 x line K2) and the hybrid pickling cucumber cultivar Tony. The F_1 hybrid plants were vigorous and gynoecious. Various expressions of dominant genes determining female sex expression were observed. Along with female flowering, some young plants formed single male flowers in the lower stem, although the middle and higher nodes as well as the branches contained only female flowers ("predominantly female"). The fruits in the F_1 generation (pickling cucumber type) were uniform with dark green skin color, marble-like, and had light small dots, thick set warts and white spines. After 30 picking dates, the F_1 plants had produced 77,760 kg/ha for marketable yield and 110,910 kg/ha for total yield. Compared to the yield of the 'Tony' control plants, this represented increases of 3.1% and 9.1%, respectively.

In the F_1 generation, five haploid plants (n = x = 7) were observed, apparently arising by gynogenesis during pollination of DH plants with line K2 pollen. These five haploid plants, when grown from seeds, were observed to be gynoecious. They developed well under greenhouse conditions, with stems reaching approximately two meters. The leaves were smaller than those of diploid plants, and the leaf blades looked almost whole with a slightly serrated periphery. The flowers were smaller in size with specific strongly cut petals (Fig. 1). Parthenocarpic fruits were formed; one of these fruits contained two very small seeds, which may have been due to mitotic division of haploid ovule cells.

The behavior of recessive genes determining main stem fasciation and the presence of lateral non-chlorophyllous branches were observed in one of the haploid plants. The stem, leaves, and fruits of this branch were light yellow.

The parthenogenesis in DH plants of line No. 50, development of haploid embryos to mature seeds in DH plants, and growth and development of plants with a haploid genome in vivo, give new knowledge about: (1) the possibility of using gynogenesis as a means of deriving and propagating of plants with a dihaploid genome; (2) interactions between female diploid or dihaploid genomes with haploid embryos; (3) vitality of cucumber haploid plants in vivo; and (4) the behavior of recessive genes determining new morphological traits.

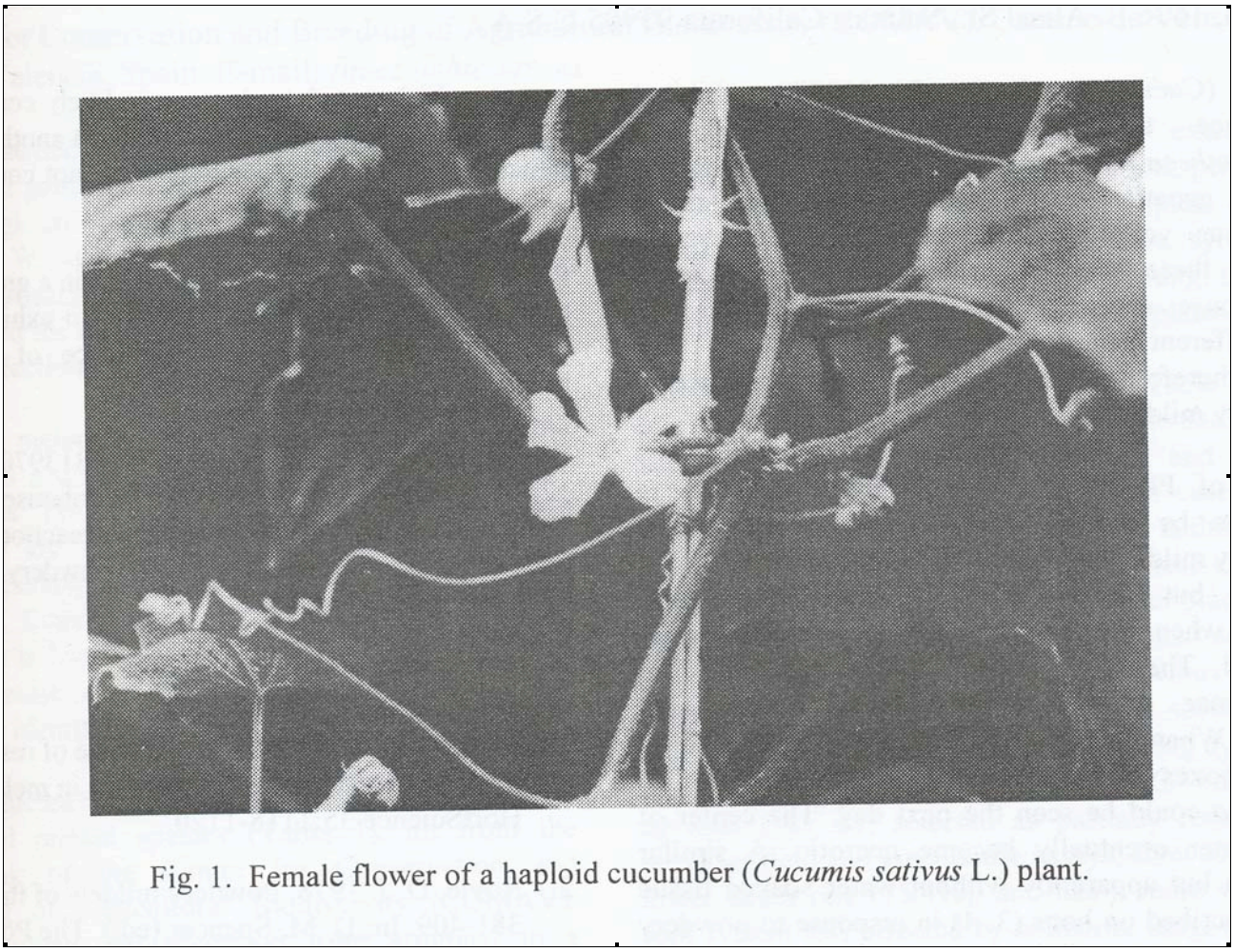

Fig. 1. Female flower of a haploid cucumber (*Cucumis sativus* L.) plant.

Fig. 1. Female flower of a haploid cucumber (*Cucumis sativus* L.) plant.

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Resistant Blister Reaction to Powdery Mildew in Melon PI 313970 and PI 124111

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Melon (*Cucumis melo* L.) PI 313970 exhibited resistance to powdery mildew incited by *Sphaerotheca fuliginea* (Schlecht ex Fr.) Poll. race 2 during genetic studies on resistance to lettuce infectious yellows virus (1). Casual observation during these studies, which were done in a greenhouse, suggested that its resistance to race 2 was different from that expressed by 'PMR 5', and might therefore possess new genes for resistance to powdery mildew.

Plants of PI 313970 were generally immune to infection by race 2 in two greenhouse tests of powdery mildew resistance in this accession (data not shown), but blister-like spots developed on some leaves when susceptible genotypes were heavily infected. The blisters were often water-soaked in appearance, or were surrounded by water-soaked tissue. When leaves with blisters were placed in moist boxes overnight, mycelia and spores of *S. fuliginea* could be seen the next day. The center of the blister eventually became necrotic. A similar reaction but apparently without water soaked tissue was described on hops (3, 4) in response to powdery mildew incited by *Sphaerotheca humuli* (D.C.) Burr. This disease reaction was first termed semi-immunity, but later called resistant blister (2). Resistant blister is characteristic of greenhouse culture of hops, but may also be exhibited in the field under high inoculum load by genotypes possessing the *B*-genotype (2).

Inheritance of resistant blister in PI 313970 is not clear. It appeared to be conditioned by a dominant gene in one greenhouse test where the resistant blister reaction data were confounded with necrotic spot and water-soaked spot data. It is more likely conditioned by a recessive gene based upon data in another test in which the resistant blister data were not confounded with other foliar reactions.

PI 124111 was, as expected, resistant in a greenhouse to race 2 (data not shown), but it also exhibited the resistant blister reaction. Inheritance of resistant blister in this accession is unknown.

Blister resistance exhibited by PI 313970 and PI 124111 may be an artifact of greenhouse culture. Comments regarding resistant blister reaction in these and other melon accessions to powdery mildew infection are welcome.

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Field Resistance to Melon Vine Decline in Wild Accessions of *Cucumis* spp. and in a Spanish Accession of *Cucumis melo*

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Melon vine decline is a complex disease with various associated pathogens (8). In south-eastern Spain, the two fungi *Acremonium cucurbitacearum* Alfaro-García, W. Gams *et* J. García-Jiménez and *Monosporascus cannonballus* Pollack *et* Uecker are considered the main causal agents of the disease, and mixed infections are frequent in this area (2).

Breeding melons for resistance or tolerance to vine decline is a difficult task. In field assays, the vine symptoms (yellowing, decay and finally plant collapse) are highly dependent on environmental factors causing water stress at the time of fruit maturity. Evaluation of root damage due to fungal infection is less influenced by environment and is a more precise indicator of resistance, and may be useful to identify resistant genotypes (3,4).

We conducted a field assay of 18 accessions of melon and wild related species (Table 1), all from the Genebank of the Center for Conservation and Breeding of Agricultural Biodiversity (COMAV). Sixteen plants per accession were arranged in a randomized complete block design with four replicates in each treatment and four plants per plot.

The assay was conducted in a field infested with *A. cucurbitacearum* and *M. cannonballus*, from which highly aggressive isolates of both fungi had been re-isolated from roots of collapsed plants in previous years. Plants were grown during the spring-summer season (planted in April, harvested in July-August). The plants were transplanted to the field at the three/four true-leaf stage.

The severity of vine decline in each plot was visually evaluated at the stage of full fruit size and fruit maturity. Several parameters were scored as follows: DR = Death rate (%), BW = Biomass weight (kg), NF = Number of fruits, FW = Fruit weight (kg). Root development and root disease severity were also scored in 2 to 6 roots per accession. RD = Root development (0 = reduced root development to 4 = vigorous, long and branched roots), RDS = Root disease severity (0 = healthy to 4 = extensively lesioned, with necrotic areas and rot roots, perithecia of *M. cannonballus*)(3,6). Root samples of the different accessions with vine decline symptoms were selected to check the presence of fungi through isolation on potato dextrose agar (PDA) plates.

The death rate varied from 0 to 100% (Table 1). The susceptible controls, cv. Amarillo Canario and Piel de Sapo, showed a mortality of 66.7 and 76.9% respectively, with a high RDS (in both cases of 4) and an intermediate RD (2 and 3). Although in previous field assays the mortality of these cultivars reached 100% (4), the lower fruit load per plant in the present assay probably contributed to plant survival. This effect has also been reported in previous studies, where the occurrence of plant collapse seems to be highly influenced by the length of growing cycle and the fruit load (7,8). The accession *C. melo* var. *agrestis* PAT 81, selected as partially resistant in previous field and greenhouse assays, showed a much lower death rate (15.4%), and had a more vigorous root system less affected by soilborne fungi (RDS=2 and RD=3). In root isolations the following fungi were detected: *M. cannonballus, Pythium* spp*, Fusarium* spp, *F. equiseti, F. oxysporum* and *F. solani.* The lack of *A. cucurbitacearum* is not surprising since this fungus is often isolated at earlier plant developmental stages (2).

Some accessions (ECU-0085, ECU-0105 (*C. dipsaceus*), UPV-05118 (*C. ficifolius*) and CA-C-25 (*C. melo*)) displayed a low percentage of mortality, from 0 to 6.3%. These species were previously reported for their resistance to *Sphaerotheca fuliginea* and *Erysiphe cichoracearum* (6). However, there is no previous report of accessions of these wild species being resistant to collapse.

Other accessions exhibited partial tolerance with a DR intermediate between the tolerant control and the susceptible controls (UPV-08629 [*C. melo*], UPV-05114 [*C. zeyheri*], UPV-05124 [*C. myriocarpus*] y UPV-08594 [*C. africanus*]).

Table 1. Analysis of variance for plant and fruit growth traits in *Cucumis* spp.accessions in a field test with high pressure from *A. cucurbitacearum* and *M. cannonballus*. Also, root growth and disease symptoms were evaluated.

Accessions	BW[z] (kg)	NF	FW(kg)	DR (%)	RD Mean	RDS Mean
Cucumis dipsaceus (ECU-0085)	1.99[y] d	88.54 e	1.91 bc	0.00	3.75±0.50	0.75±0.50
C. dipsaceus (ECU-0105)	1.65 bcd	98.56 e	2.82 c	0.00	3.00±1.73	0.33±0.58
C. ficifolius (UPV-05118)	1.86 cd	40.00 bcd	0.82 ab	0.00	4.00±0.00	1.00±1.41
C. melo (CA-C-25)	0.86 ab	0.44 a	0.39 ab	6.25	2.83±0.75	2.83±1.47
C. melo var *agrestis* PAT 81	1.00 abc	8.58 ab	2.60 c	15.38	3.00±0.00	2.00±1.09
C. myriocarpus (UPV-05124)	0.98 abc	43.17 cd	0.30 ab	16.67	2.33±1.15	2.33±1.71
C. zeyheri (UPV-05114)	0.12 a	5.10 ab	0.12 a	20.00	1.00±0.00	2.75±0.95
C. africanus (UPV-08594)	0.07 a	3.46 a	0.07 a	33.33	1.00±0.00	4.00±0.00
C. melo (UPV-08629)	0.97 abc	4.40 ab	2.95 c	36.36	3.50±0.71	3.00±0.00
C. anguria var *longipes* (UPV-05125)	0.67 a	23.37 abc	0.52 ab	50.00	2.50±0.55	4.00±0.00
C. melo (C-C-30)	0.34 a	1.00 a	0.59 ab	66.67	2.00±0.00	4.00±0.00
C. melo (V-C-146)	0.41 a	1.13 a	1.10 ab	66.67	2.00±0.00	4.00±0.00
C. melo (Amarillo Canario)	0.49 a	0.31 a	0.16 a	66.67	2.00±0.00	4.00±0.00
C. melo (V-C-184)	0.27 a	0.31 a	0.14 a	71.43	-	4.00±0.00
C. anguria (UPV-05162)	0.35 a	65.37 de	0.28 a	75.00	3.00±0.00	3.50±0.71
C. melo (Piel de Sapo)	0.62 a	0.87 a	0.29 a	76.92	3.00±0.00	4.00±0.00
C. melo (C-C-34)	0.51 a	1.14 a	0.70 ab	80.00	3.00±0.00	4.00±0.00
C. anguria var *longipes* (UPV-05126)	0.18 a	28.92 abc	0.43 ab	100.00	1.00±0.00	4.00±0.00

P				
	Accessions	0.0001*	0.0000*	0.0000*
	Block	0.4072	0.0122*	0.0040*

[z]BW-Biomass weight, NF-number of fruits, FW-fruit weight,DR-death rate, RD- root development (0 = reduced root development, 4 = vigorous, branched roots), RDS-root disease severity (0 = healthy, 4 = extensive lesions, perithecia of *M. cannonballus*).

[y]Means followed by the same letter are not significantly different at the 5% level, Duncans mean comparison.

The accession CA-C-25 (*C. melo*) had a DR lower than the resistance source, PAT 81 exhibited a RDS of 2.83, similar to that of PAT 81 and a root disease severity of 2.83, slightly higher than that of PAT 81. This accession is also interesting because it has resistance to powdery mildew. It is possible that the high incidence of powdery mildew increased the death rate of *C. melo* var. *agrestis* PAT 81, while the accession CA-C-25 was not affected by the disease. CA-C-25 is a cultivated type, very similar to Galia melons. After some selection, it could be used directly in field, and also, it would be simple to incorporate its resistance in other types of melon. In any case it is necessary to check the tolerance to vine decline under artificial inoculation conditions

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Fusarium Wilt Resistance in Eight Identified Multiple Disease Resistant Genotypes of *Cucumis melo* L.

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In an ongoing study to identify multiple-disease resistant (MDR) genotypes and accessions as a step towards incorporating these genotypes as donor parents in the improvement of existing cultivars (1), the present study attempted to isolate and identify the wilt causing pathogen from nursery raised 'Pusa Madhuras' (PM) seedlings. Pathogenicity of a pure *Fusarium oxysporum* f. sp. *melonis* isolate, the wilt causing pathogen in *Cucumis melo*, has been established which causes yellowing, necrosis and finally wilting under conditions of artificial inoculation. *Fusarium* wilt of melons occurs worldwide (3,7), and Risser, et al. in 1976 (4) proposed four races of the pathogen (Races 0, 1, 2, and 1-2). However, race identification (3,4) has not been possible at IARI so far.

Materials and Methods: *Plant materials.* Standardized cropping practices were used to grow melon accessions and cultivars in the field in order to generate seed for artificial screening against *Fusarium* sp. Cultivars and accessions used included PM and eight identified multiple disease resistant (MDR) melon genotypes which exhibited *Fusarium* wilt field resistance ('Honeydew', MR-1, 'Nantais-Oblong', 'Ogon-9', PMR-5, PI 414723, 'Topmark bush', and WMR-29). The MDR genotypes were selected based on their field resistance to major diseases like powdery mildew, viruses (e.g. CGMMV) and *Fusarium* wilt (PDI <25%) at various stages of development up to harvest during 1997-2000

Isolation, identification and purification of wilt causing local strain of Fusarium sp.: PM seedlings were grown in polythene bags in March, 1998. The soil mixture (FYM:sand:soil in 1:1:1 ratio) was not treated with captan. Wilting was observed in 55% of the germinated seedlings at the 4-5 true leaf stage. Wilt symptoms first appeared in the root zone, followed by cotyledonary leaves and the first pair of true leaves. The wilt causing pathogen was found to be *Fusarium* sp. The pathogen was isolated, identified, purified, and maintained as a pure isolate. PM seedlings were also grown in captan drenched soil to identify any other incidences of wilting or pathogen activity.

Pathogenicity test: *Fusarium* cultures 7-8 days old were used for the pathenogicity test. Cultures with abundant mycelia were filtered through a muslin cloth before adjusting the spore density with autoclaved distilled water to 2 x 10^5 spores/g of soil mixture (5). Artificial screening was conducted by inoculating the soil around the root zone of PM seedlings with varying spore densities. Percent disease incidence (PDI) was calculated, and spore densities which resulted in >50% and 100% PDI for PM were determined for further screening.

Preparation of seedlings: Melon seeds were surface sterilized with 0.1% $HgCl_2$ for 10 min, then washed thoroughly with autoclaved distilled water. Disinfested seeds were sown in an autoclaved soil mixture in sterilized trays, in four rows of 10-20 seeds each. After germination, two rows of 7-8 days old seedlings were inoculated with pure, pathogenic, *Fusarium* spores at densities of 1.36 x 10^5, 2.0 x 10^5 , and 2.7 x 10^5 spores/g of soil for artificial screening. The remaining two rows of uninoculated seedlings served as the control. Screening was done in open field conditions under a glass cover after inoculation.

Reaction of MDR genotypes to Fusarium isolate: Genotypes were classified as *Fusarium* resistant (FR; <25% PDI), *Fusarium* moderately resistant (FMR; 25-50% PDI) and *Fusarium* susceptible (FS; >50% PDI) on the basis of mortality as evidenced by yellowing, necrosis and finally wilting of cotyledons/seedlings 8-10 days after inoculation using a spore density of 2 x 10^5 spores/g of soil mixture (5). Uninoculated and healthy green seedlings had generally reached the flowering stage by this time..

Results and Discussion: Captan drenching provided a chemical control measure to *Fusarium* wilt in melons. No *Fusarium* was detected from captan drenched soil in the field. Similarly no *Fusarium* could be detected from captan drenched, nursery

Table 1: Effect of spore concentration on *Fusarium* pathogenicity for cv. Pusa Madhuras (PM) seedlings.

Spores density (spores/gm of soil mixture)	PDI[z]
0 (control)	5
1.36×10^5	50
2.0×10^5	73 ± 9
2.7×10^5	100

[z]PDI: Percent disease incidence

Table 2. Artificial screening of eight identified resistant accessions to *Fusarium* wilt by soil inoculation method.

Accessions	Percent disease incidence (PDI)		Class
	Control (no Fusarium)[z]	Inoculated conditions[y]	
Honeydew	0.0*	23.61 ± 1.96	FR
MR-1	0.0	16.25 ± 5.30	FR
Nantais-Oblong	0.0	0.0	FR
Ogon-9	0.0	17.14 ± 4.04	FR
PMR-5	0.0	0.0	FR
P1-4 14723	0.0	5.55 ± 6.03	FR
Topmark Bush	0.0	0.0	FR
WMR-29	0.0	6.25 ± 6.73	FR

[z] Control: autoclaved soil with no *Fusarium* inoculation.
[y] Autoclaved soil with *Fusarium* inoculation of spores density as 1.36×10^5 spores/g of soil mixture.
[x] 'Honeydew' exhibited some cotyledonary shriveling.

raised PM seedlings and plants, although *Pythium* wilting was observed in 3% of the PM plants, which wilted at 2-4 true leaf stage.

The pure *F. oxysporum* isolate was found to be highly pathogenic, and could induce 73% PDI with a spore density 2 x 10^5 spores/g of soil mixture on PM under artificial inoculation conditions. No *Fusarium* incidence was observed in 95% of the uninoculated control PM seedlings.

A spore density of 1.36 x 10^5 spores/g of soil mixture (which caused 50% PDI in PM) was used for artificial screening of the 8 MDR genotypes. PDI was <25% for all 8 genotypes (Table 2), and they were therefore classified as *Fusarium* resistant genotypes by the artificial screening method. Previously, Zuniga and Zitter (7) had confirmed MR-I as FR based on artificial screening after establishing the pathogenicity of their isolate

Similar studies have identified *Fusarium* wilt as a significant factor in melon production (2,6,7). The 8 identified FR genotypes of *C. melo* can be incorporated as donor parents in the resistance breeding program of existing cultivars. In addition, molecular marker techniques may be useful in the future to utilize the gene(s) that control resistance to *Fusarium* wilt.

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Inheritance of RAPD Markers in Melon (*Cucumis melo* L.)

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Introduction: Genetic markers have been employed in diversity analysis (2,3,4,7,8) and the construction of maps in melon (1,9). The use of random amplified polymorphic DNA (RAPD) has allowed for discrimination of elite (2,7) and unadapted germplasm (3,4,8). Because of their relative low cost and low technological attributes they have been valuable for diversity analysis (2,7). However, except for the mapping of several RAPD marker loci by Baudracco-Arnas and Pitrat (1996), the genetics RAPD markers in melon has not been widely characterized. This is likely due to the relatively low level of RAPD polymorphisms (~15-20%) in melon. The bands used for diversity analysis are repeatable (4,7), but their genetic attributes have not been characterized. This report details the genetics of RAPD markers assessed in F_2 progeny segregating in four melon populations.

Materials and Methods: Crosses were made between an experimental inbred line SA 200 ('Chargyne') x 'Top Mark', WI 998 x 'Top Mark', Charentais-1 x AR5, and 'Top Mark' x AR5. These crosses were made to produce segregating populations that would be useful in mapping disease resistance and sex expression in melon. SA 200 is a gynoecious Charentais market type received from Clause Seed Company (Bretigny-Sur-Orge Cedex, France), Charentais-1 is a gynoecious Charentais market type inbred line received from Petoseed Company (now associated with Seminis, Woodland Calif., USA), AR5 and "Top Mark' are disease resistant U.S. Western Shipper market types released by the USDA, ARS, and WI 998 is a gynoecious line released by the USDA, ARS. Each F_1 was self-pollinated to produce four F2 populations (i.e., SA 200 x 'Top Mark', WI 998 x 'Top Mark', Charentais-1 x AR5, and 'Top Mark' x AR5) segregation analyses.

DNA was extracted and subjected to PCR using RAPD primers from Operon (Alameda, Calif., USA) and University of British Columbia (UVBC; designated BC; Vancouver, BC, Canada) and electrophoresis was carried out according to Staub et al. (7). Initially, a survey of all parents was made using about 1,500 primers to identify polymorphism specific to the contrasting parents in a particular cross. Primer products were identified by their primer designation (e.g., B12 = Operon primer and BC541 = UVBC primer) (Table 1). When a primer produced more than one product that was useable for analysis, it was given a lower case letter designation from cathodal to anodal migration position (e.g., BC541a).

Data were obtained for dominant RAPD loci from F_2 families and analyzed by chi-square analysis for conformity to expected 3:1 (df = 1) Mendelian single-factor segregation ratios.

Results and Discussion: The primers examined yielded between 25 to 40% polymorphisms (band differences between parents) depending on the cross (data not presented). Potentially useful bands were characterized as having a mobility between 200 to 2,500 bp. Putative loci were then identified as those possessing bands that were reproducible and bright and used for segregation analysis (Table 1). Initially, examination of parents of SA 200 x 'Top Mark', WI 998 x 'Top Mark', Charentais-1 x AR5, and 'Top Mark' x AR5 produced 264, 241, 244, and 251 reproducible band differences between respective parents (putative loci) (data not presented). On average, this represents 17% recovery of potentially useable bands (loci) from the survey of 1,500 primers.

After this initial assessment, 41, 27, 90, and 38, bands (loci) segregated in a predictable manner in progeny from SA 200 x 'Top Mark', WI 998 x 'Top Mark', Charentais-1 x AR5, and 'Top Mark' x AR5 matings, respectively (Table 1). This represents, on average, 3.3% recovery of useable loci from the initial survey of 1,500 primers.

Given the fact that individuals are sometimes misclassified even when scoring loci that historically have proven to reproducible bright RAPD bands, we are attempting RAPD to SCAR conversion at some

Table 1. F_2 single factor segregation for RAPD primer products[z] in melon (*Cucumis melo* L.).

Cross	Primer	bp	No. F2	Obs	Exp	Chi. sq	*P* value>
SA 200	M7	675	76	57	57	NA	NA
x TM	U13	831	76	57	57	NA	NA
	AT7a	1275	76	57	57	NA	NA
	AV11	815	79	59	59.25	0.00	0.95
	D16	1850	77	58	57.75	0.00	0.95
	AB17	831	78	59	58.5	0.00	0.95
	AC7a	450	78	58	58.5	0.00	0.95
	AG15	975	78	58	58.5	0.00	0.95
	R11	1100	78	59	58.5	0.00	0.95
	U10	870	78	59	58.5	0.00	0.95
	AV11	1900	74	55	55.5	0.00	0.95
	Y13	610	74	56	55.5	0.00	0.95
	AV1	1375	70	53	52.5	0.00	0.95
	K4	564	50	37	37.5	0.01	0.70
	I4b	831	71	54	53.25	0.01	0.70
	E8	1300	76	58	57	0.02	0.70
	M7	650	76	56	57	0.02	0.70
	W3a	1000	75	55	56.25	0.03	0.70
	R19b	564	75	55	56.25	0.03	0.70
	C13	1275	73	56	54.75	0.03	0.70
	Y15	1050	71	52	53.25	0.03	0.70
	AM14a	1500	78	60	58.5	0.04	0.70
	Y13	575	74	54	55.5	0.04	0.70
	P6	1200	79	61	59.25	0.05	0.70
	AE3a	1250	75	58	56.25	0.05	0.70
	AL9	1650	75	58	56.25	0.05	0.70
	E8	675	76	59	57	0.07	0.70
	AH2b	500	76	55	57	0.07	0.70
	C20	700	76	55	57	0.07	0.70
	AB4b	1325	45	32	33.75	0.09	0.70
	AI11	600	78	56	58.5	0.11	0.70
	W10c	830	78	56	58.5	0.11	0.70
	AA14	1375	74	53	55.5	0.11	0.70
	AG2	1375	74	53	55.5	0.11	0.70
	B11	1400	80	63	60	0.15	0.10
	AK5b	1500	78	62	58.5	0.21	0.10
	G6	1890	78	55	58.5	0.21	0.10
	AF7	1370	70	49	52.5	0.23	0.10
	G8	1375	79	63	59.25	0.24	0.10
	L1	564	79	63	59.25	0.24	0.10
	AJ12	750	75	60	56.25	0.25	0.10
WI 998	A16	1600	92	69	69	NA	NA
x TM	AT2	800	84	63	63	NA	NA
	BC226	1400	92	69	69	NA	NA
	O2-1	1100	88	66	66	NA	NA
	AF7	947	91	68	68.25	0.00	0.95
	AL8-1	820	75	56	56.25	0.00	0.95
	E6-1	1100	86	64	64.5	0.00	0.95
	AH9-1	1050	78	58	58.5	0.00	0.95
	Z9	2050	78	59	58.5	0.00	0.95
	AB4-2	1300	93	69	69.75	0.01	0.70
WI 998	AK5	800	93	69	69.75	0.01	0.70
x TM	M7-2	750	87	66	65.25	0.01	0.70
	AO8	1600	84	64	63	0.02	0.70
	A11-1	960	76	56	57	0.02	0.70
	AI8-1	800	89	68	66.75	0.02	0.70
	AT7-1	860	87	64	65.25	0.02	0.70
	AD12	1100	86	66	64.5	0.03	0.70
	AB16-1	1375	78	57	58.5	0.04	0.70
	AJ20	800	92	71	69	0.06	0.70
	J7	1600	92	67	69	0.06	0.70
	AG13	975	88	68	66	0.06	0.70
	AI9	520	93	72	69.75	0.07	0.70
	J7	700	93	72	69.75	0.07	0.70
	AB16	1400	87	63	65.25	0.08	0.70
	AH3-2	1700	87	63	65.25	0.08	0.70
	AT7-1	950	85	66	63.75	0.08	0.70
	V1-1	564	82	59	61.5	0.10	0.70
Charent.	Z3a	700	92	69	69	NA	NA
x AR5	D16	2200	93	70	69.75	0.00	0.95
	F1	1100	93	70	69.75	0.00	0.95
	O19a	1200	93	70	69.75	0.00	0.95
	AB4b	1350	93	70	69.75	0.00	0.95
	AT2b	1000	93	70	69.75	0.00	0.95
	AU19	580	93	70	69.75	0.00	0.95
	BC299	700	93	70	69.75	0.00	0.95
	AU2a	1400	90	67	67.5	0.00	0.95
	BC628	830	90	68	67.5	0.00	0.95
	I4	970	93	69	69.75	0.01	0.95
	AB8	1350	93	69	69.75	0.01	0.95
	AT15b	1890	91	69	68.25	0.01	0.95
	H2	600	89	66	66.75	0.01	0.95
	C20	1000	93	71	69.75	0.02	0.70
	D9a	800	93	71	69.75	0.02	0.70
	E6	700	93	71	69.75	0.02	0.70
	U13	1000	93	71	69.75	0.02	0.70
	AA12	830	93	71	69.75	0.02	0.70
	R5a	1000	91	67	68.25	0.02	0.70
	AV4	2027	91	67	68.25	0.02	0.70
	AB17	1000	44	32	33	0.03	0.70
	R11	1300	90	69	67.5	0.03	0.70
	S4	1910	93	68	69.75	0.04	0.70
	W10	575	93	68	69.75	0.04	0.70
	AF12b	800	93	68	69.75	0.04	0.70
	AL9a	1890	93	68	69.75	0.04	0.70
	C10	900	92	71	69	0.06	0.70
	AU2b	700	92	67	69	0.06	0.70
	AB1	831	93	72	69.75	0.07	0.70
	AD12	1000	93	72	69.75	0.07	0.70
	AM1	950	93	72	69.75	0.07	0.70
	AP2	1375	93	72	69.75	0.07	0.70
	BC541a	2100	93	72	69.75	0.07	0.70

Cross	Primer	bp	No. F2	Obs	Exp	Chi. sq	*P* value>
Charent. x AR5	B11	950	93	67	69.75	0.11	0.70
	N11a	1375	93	67	69.75	0.11	0.70
	Y10	775	93	67	69.75	0.11	0.70
	AF7b	831	93	67	69.75	0.11	0.70
	AH20	500	93	67	69.75	0.11	0.70
	AJ17	700	93	67	69.75	0.11	0.70
	AK3	500	92	66	69	0.13	0.70
	L15	800	93	73	69.75	0.15	0.70
	Y15	1000	93	73	69.75	0.15	0.70
	AF20c	580	93	73	69.75	0.15	0.70
	AG4a	1400	93	73	69.75	0.15	0.70
	AT2a	1100	93	73	69.75	0.15	0.70
	BC388	1100	93	73	69.75	0.15	0.70
	J4	831	93	66	69.75	0.20	0.50
	AG10a	530	93	66	69.75	0.20	0.50
	AL8b	400	93	66	69.75	0.20	0.50
	L1	800	91	72	68.25	0.21	0.50
	Q10	1580	91	72	68.25	0.21	0.50
	AK5	800	78	62	58.5	0.21	0.50
	AV11	1000	89	63	66.75	0.21	0.50
	B14	1400	92	65	69	0.23	0.50
	F3	400	92	73	69	0.23	0.50
	AX16	1100	92	65	69	0.23	0.50
	D9b	750	93	74	69.75	0.26	0.50
	J7c	400	93	74	69.75	0.26	0.50
	BC654	1000	93	74	69.75	0.26	0.50
	U8	974	93	65	69.75	0.32	0.50
	AE2b	550	93	65	69.75	0.32	0.50
	AF20a	2300	93	65	69.75	0.32	0.50
	AJ12	800	93	65	69.75	0.32	0.50
	AT15a	2000	91	73	68.25	0.33	0.50
	L11	1110	92	64	69	0.36	0.50
	C13	1375	93	75	69.75	0.40	0.50
	K4	700	93	75	69.75	0.40	0.50
	N11b	1000	93	75	69.75	0.40	0.50
	U1	1904	93	75	69.75	0.40	0.50
	AT2c	780	93	75	69.75	0.40	0.50
	BC388	1000	93	75	69.75	0.40	0.50
	O19c	400	93	64	69.75	0.47	0.30
	AF7a	840	93	64	69.75	0.47	0.30
	AM19	1900	89	61	66.75	0.50	0.30
	AL9b	600	93	76	69.75	0.56	0.30
	AF20b	1375	93	77	69.75	0.75	0.30
	AG4b	700	93	77	69.75	0.75	0.30
	X17	700	67	44	50.25	0.78	0.30
	X19	1910	93	62	69.75	0.86	0.30
	A17b	900	87	73	65.25	0.92	0.30
	Z3b	600	92	61	69	0.93	0.30
	AI14	700	93	78	69.75	0.98	0.30
	AN1	800	93	78	69.75	0.98	0.30
	J7b	775	93	61	69.75	1.10	0.20
Charent. x AR5	AF7c	600	93	79	69.75	1.23	0.20
	T17a	1910	78	49	58.5	1.54	0.20
	AF12a	1000	93	59	69.75	1.66	0.10
	U7	1050	87	52	65.25	2.69	0.10
TM x AR5	K4 c	831	43	32	32.25	0.00	0.95
	E6	960	62	47	46.5	0.01	0.95
	Z11 b	300	42	31	31.5	0.01	0.95
	X16	575	38	29	28.5	0.01	0.95
	AM18	831	22	16	16.5	0.02	0.70
	AF7	831	45	33	33.75	0.02	0.70
	AG15	974	45	33	33.75	0.02	0.70
	F1	2027	43	33	32.25	0.02	0.70
	Z18	831	43	33	32.25	0.02	0.70
	BC388	1090	76	56	57	0.02	0.70
	AX19	300	98	72	73.5	0.03	0.70
	W7	831	40	29	30	0.03	0.70
	AX20	900	42	33	31.5	0.07	0.70
	BC526	825	39	31	29.25	0.10	0.70
	AJ20	1375	44	31	33	0.12	0.70
	AT3	1570	44	35	33	0.12	0.70
	AV11 b	831	40	28	30	0.13	0.70
	AF20	1400	22	15	16.5	0.13	0.70
	AP2	1100	43	30	32.5	0.15	0.70
	AV11 a	960	72	57	54	0.16	0.50
	K4 a	975	42	29	31.5	0.19	0.50
	AQ6	947	45	31	33.75	0.22	0.50
	H2	825	43	35	32.25	0.23	0.50
	AO18 a	1800	43	35	32.25	0.23	0.50
	BC299	700	40	33	30	0.30	0.50
	U5	564	45	37	33.75	0.31	0.50
	U10	835	45	37	33.75	0.31	0.50
	O6	625	42	28	31.5	0.39	0.50
	T1	947	69	57	51.15	0.53	0.30
	AX6 a	575	45	29	33.75	0.67	0.30
	W10	835	74	50	55.5	0.55	0.30
	Z11 a	1584	43	37	32.25	0.69	0.30
	AD12	1000	73	61	54.75	0.71	0.30
	E1	795	76	50	57	0.86	0.30
	L2	1000	42	37	31.5	0.96	0.30
	AB3	835	42	37	31.5	0.96	0.30
	Q10 a	1800	73	45	54.75	1.74	0.10
	Z8	831	44	41	33	1.94	0.10

[z] Products designated as primer and lower case letter (e.g., AC7a) (NA = not applicable).

of the loci (e.g., $M7_{675}$, $U13_{831}$, $AT7a_{1275}$, $A16_{1600}$, $AT2_{800}$, $BC226_{1400}$, $02\text{-}1_{1100}$, and $Z3a_{700}$). Although this type of conversion has proven difficult in cucumber (6), if success is achieved in melon we will make additional conversions. This will allow for the development of a standard array of SCARs markers, and permit their use in diversity analysis and genetic map construction along with previously published codominant markers (5). A standard marker array and the use of reference accessions from previous studies (e.g., 7) will provide powerful set of tools for diversity analysis.

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Frequency Differences of RAPD Markers in Market Classes of Melon (*Cucumis melo* L.)

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Introduction: The determination of variability in *Cucumis melo* L. market classes is of importance to germplasm management, plant variety protection, and in the development of breeding strategies. The genetic diversity of several commercially important melon groups (principally Cantaloupensis and Inodorus) has been characterized using molecular analyses (4, 3, 6). Simple sequence repeat (SSR) and random amplified polymorphic DNA (RAPD) markers have been used to differentiate elite melon germplasm (1, 2).

Staub et al. (5) used RAPD and SSR markers to characterize genetic relationships among 46 melon accessions in two *C. melo* L. subsp. *melo* (Cantaloupensis, Inodorus) and subsp. *agrestis* (Conomon and Flexuosus) groups. They examined genetic variation in accessions of diverse market classes of Cantaloupensis (Charentais, European and U.S. Western Shipper, U.S. Eastern Market, Galia, and Ogen) and Inodorus [Honeydew, and Casaba (syn. Spanish; Rochet, Piel de Sapo, and Amarillo)]. We provide herein a summary of the variation of Group Flexuous and Conomon accessions and the Cantaloupensis and Inodorus major market class accessions examined by Staub et al. (5) grouped by RAPD marker. Such a summary will allow researchers to development a RAPD marker array(s) to best suit their needs for strategic analyses of germplasm.

Materials and Methods: RADP profiling data of Charentais (7), European (6), U.S. Western Shipper (3), U.S. Eastern Market (4), Galia (7), Ogen (6), Honeydew (2), Casaba (9), group Conomon (1), and group Flexuosus (1) accessions used by Staub et al. (5) were taken collectively. These accessions originated from seed companies (5) and the U.S. Department of Agriculture, Agricultural Research Service.

Data were provided by 57 RAPD primers (Operon and University of British Columbia (BC)] producing 118 RAPD bands (Table 1). A marker was considered repeatable if PCR yielded a consistent result in all of three (or more) replications (putative loci; see companion paper this issue). Each RAPD marker was named by the primer designation followed by an upper case letter. Tabulations summarize the percentage of RAPD band presence within a market class or subspecies and among European and U.S. germplasm as an estimation of the polymorphism level and diversity within groups. This was calculated as number of accessions with band presence divided by the total number of accessions examined and then multiplied by 100. This calculation is hereafter referred as percent frequency.

Results and Discussion: The vast majority of RAPD markers were found to have a similar percent frequency in accessions in Europe and USA. Only one marker appeared completely absent in USA accessions (L18-B) and two markers were absent in European accessions (AO8-A and AS14-B) (Table 1).

All groups showed a similar average percent frequency across markers. Given the relatively large standard deviations from mean values, the variation in all the market classes examined was relatively large. The information presented regarding geographic regions may, however, depend on the germplasm array analyzed. For instance, some markers (e.g. AT5-C, AS14-A, AG15-B, BC226-B, BC407-B, and BC526-A) were present in all accessions but not in Conomon group. Likewise, some markers were absent in all accessions but not in a few groups (e.g. AO8-A). Some primers (e.g. C1, F1, F4, AO8, BC231, BC280, BC403, BC617, and BC663) produced products that provided minimal information for discrimination. One primer, BC252, was particularly not informative for characterizing differences in group Inodorus. Nevertheless, the great majority of polymorphisms was observed in this study provide for adequate variation to elucidate within and among group differences.

Table 1. Percentage of RAPD band presence within a market class or subspecies (Inodorus, Cantalupensis, Conomon, and Flexuosus) and among European and U.S. germplasm.

	Inodorus		Cantaloupensis									
Primer	Casaba	Honeydew	Charentais	European *Shipper*	Galia	Ogen	U.S. Eastern Market	U.S. Western Shipper	Conomon	Flexuosus	Europe	USA
B12-A[z]	22.2	0.0	57.1	100.0	42.9	16.7	100.0	66.7	100.0	100.0	43.8	71.4
B12-B	33.3	50.0	85.7	100.0	42.9	100.0	100.0	100.0	100.0	0.0	68.8	78.6
C1-A	100.0	100.0	100.0	100.0	57.1	100.0	100.0	100.0	100.0	0.0	93.8	85.7
D7-A	44.4	0.0	0.0	0.0	85.7	100.0	0.0	0.0	0.0	0.0	40.6	21.4
D7-B	100.0	100.0	42.9	100.0	71.4	100.0	75.0	100.0	100.0	100.0	84.4	85.7
D7-C	88.9	50.0	100.0	100.0	57.1	16.7	100.0	100.0	100.0	100.0	68.8	100.0
D7-D	100.0	100.0	85.7	100.0	71.4	100.0	100.0	100.0	100.0	100.0	90.6	100.0
F1-A	100.0	100.0	100.0	83.3	100.0	100.0	100.0	66.7	100.0	100.0	96.9	92.9
F4-A	100.0	100.0	85.7	100.0	57.1	50.0	100.0	100.0	100.0	100.0	81.3	92.9
F4-B	88.9	100.0	100.0	100.0	71.4	100.0	100.0	100.0	100.0	100.0	93.8	92.9
G8-A	33.3	50.0	14.3	0.0	0.0	0.0	0.0	0.0	100.0	0.0	15.6	7.1
G8-B	100.0	100.0	85.7	100.0	71.4	16.7	100.0	66.7	0.0	100.0	78.1	78.6
I4-A	33.3	100.0	42.9	66.7	57.1	83.3	100.0	100.0	100.0	100.0	56.3	85.7
I4-B	100.0	100.0	57.1	100.0	71.4	16.7	75.0	100.0	100.0	100.0	68.8	92.9
I16-A	11.1	0.0	14.3	16.7	14.3	0.0	25.0	66.7	0.0	0.0	9.4	28.6
I16-B	100.0	50.0	85.7	100.0	100.0	100.0	100.0	100.0	0.0	100.0	93.8	92.9
L18-A	88.9	50.0	100.0	100.0	71.4	60.0	100.0	66.7	0.0	100.0	87.1	71.4
L18-B	0.0	50.0	0.0	0.0	0.0	16.7	0.0	0.0	0.0	0.0	6.3	0.0
L18-C	66.7	100.0	100.0	83.3	42.9	66.7	50.0	100.0	0.0	100.0	71.9	71.4
N6-A	100.0	100.0	100.0	100.0	71.4	100.0	100.0	100.0	100.0	100.0	93.8	100.0
N6-B	77.8	50.0	100.0	100.0	57.1	16.7	100.0	100.0	100.0	100.0	71.9	85.7
N6-C	11.1	0.0	42.9	0.0	28.6	16.7	0.0	0.0	0.0	0.0	18.8	7.1
W7-A	88.9	100.0	100.0	66.7	42.9	0.0	100.0	100.0	100.0	100.0	65.6	85.7
W7-B	88.9	50.0	14.3	16.7	14.3	0.0	0.0	33.3	100.0	0.0	28.1	35.7
AB14-A	88.9	100.0	85.7	100.0	85.7	100.0	100.0	66.7	0.0	0.0	96.9	64.3
AB14-B	100.0	0.0	28.6	16.7	28.6	0.0	0.0	33.3	0.0	100.0	34.4	35.7
AD12-A	33.3	50.0	71.4	100.0	85.7	100.0	100.0	100.0	0.0	100.0	71.9	85.7
AD14-A	77.8	0.0	0.0	33.3	42.9	50.0	25.0	0.0	0.0	100.0	40.6	28.6
AE6-A	33.3	50.0	85.7	33.3	28.6	50.0	25.0	100.0	100.0	100.0	43.8	64.3
AE6-B	66.7	50.0	14.3	16.7	57.1	0.0	75.0	0.0	0.0	0.0	34.4	35.7
AF7-A	77.8	100.0	28.6	100.0	71.4	100.0	75.0	66.7	100.0	100.0	75.0	78.6
AF7-B	100.0	50.0	100.0	100.0	100.0	100.0	50.0	66.7	0.0	100.0	96.9	71.4
AF7-C	77.8	0.0	85.7	50.0	85.7	0.0	75.0	0.0	0.0	100.0	56.3	57.1
AF14-A	77.8	100.0	28.6	83.3	57.1	100.0	100.0	66.7	0.0	100.0	71.9	71.4
AG15-A	11.1	0.0	14.3	100.0	0.0	0.0	25.0	100.0	100.0	0.0	21.9	42.9
AG15-B	88.9	100.0	100.0	83.3	100.0	100.0	100.0	100.0	100.0	100.0	96.9	92.9
AG15-C	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	92.9
AG15-D	100.0	50.0	28.6	33.3	14.3	0.0	0.0	66.7	0.0	100.0	37.5	42.9
AJ18-A	44.4	0.0	85.7	100.0	14.3	16.7	100.0	100.0	0.0	100.0	46.9	78.6
AJ18-B	77.8	100.0	57.1	100.0	85.7	100.0	100.0	66.7	100.0	100.0	87.5	78.6

	Inodorus		Cantaloupensis									
Primer	Casaba	Honeydew	Charentais	European *Shipper*	Galia	Ogen	U.S. Eastern Market	U.S. Western Shipper	Conomon	Flexuosus	Europe	USA
AK16-A	100.0	100.0	85.7	100.0	100.0	100.0	100.0	66.7	0.0	100.0	96.9	85.7
AL5-A	77.8	0.0	100.0	0.0	14.3	0.0	25.0	0.0	100.0	100.0	37.5	42.9
AM2-A	88.9	100.0	57.1	83.3	71.4	33.3	100.0	33.3	100.0	100.0	65.6	85.7
AN5-A	100.0	0.0	85.7	66.7	85.7	100.0	100.0	100.0	0.0	100.0	81.3	92.9
AN5-B	100.0	0.0	100.0	0.0	71.4	100.0	0.0	33.3	100.0	100.0	71.9	50.0
AN5-C	22.2	100.0	85.7	83.3	71.4	0.0	100.0	100.0	100.0	0.0	53.1	78.6
AO8-A	11.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.1
AO8-B	88.9	100.0	85.7	100.0	57.1	100.0	100.0	100.0	100.0	100.0	87.5	92.9
AO19-A	88.9	50.0	28.6	50.0	85.7	100.0	100.0	33.3	100.0	100.0	68.8	78.6
AO19-B	100.0	100.0	100.0	100.0	85.7	100.0	100.0	100.0	0.0	100.0	96.9	92.9
AS14-A	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	92.9
AS14-B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	100.0	100.0	0.0	21.4
AS14-C	100.0	100.0	100.0	100.0	71.4	100.0	100.0	100.0	100.0	100.0	96.9	92.9
AS14-D	100.0	0.0	100.0	0.0	85.7	100.0	50.0	0.0	0.0	100.0	71.9	57.1
AT1-A	88.9	50.0	57.1	83.3	42.9	100.0	100.0	66.7	0.0	100.0	71.9	78.6
AT2-A	88.9	100.0	71.4	66.7	100.0	100.0	75.0	66.7	0.0	100.0	87.5	71.4
AT2-B	66.7	100.0	57.1	100.0	28.6	0.0	50.0	100.0	0.0	100.0	56.3	57.1
AT2-C	100.0	50.0	100.0	83.3	71.4	100.0	75.0	66.7	0.0	100.0	87.5	78.6
AT5-A	33.3	50.0	57.1	0.0	28.6	50.0	50.0	33.3	100.0	100.0	34.4	50.0
AT5-B	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	92.9
AT7-A	33.3	50.0	85.7	100.0	57.1	100.0	75.0	100.0	100.0	100.0	75.0	71.4
AT15-A	55.6	100.0	14.3	83.3	71.4	100.0	100.0	100.0	0.0	100.0	68.8	71.4
AU2-A	11.1	50.0	14.3	100.0	28.6	0.0	100.0	33.3	100.0	0.0	34.4	42.9
AU2-B	66.7	100.0	100.0	0.0	28.6	16.7	100.0	0.0	100.0	100.0	50.0	57.1
AU2-C	0.0	0.0	28.6	0.0	0.0	0.0	25.0	66.7	100.0	0.0	6.3	28.6
AV11-A	77.8	50.0	100.0	33.3	100.0	100.0	25.0	0.0	0.0	100.0	78.1	50.0
AV11-B	77.8	50.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	90.6	92.9
AV11-C	66.7	100.0	85.7	100.0	85.7	100.0	100.0	0.0	0.0	100.0	87.5	64.3
AV11-D	22.2	50.0	71.4	16.7	100.0	66.7	100.0	0.0	0.0	100.0	50.0	64.3
AW10-A	100.0	100.0	85.7	100.0	85.7	100.0	100.0	100.0	0.0	100.0	93.8	92.9
AW10-B	11.1	0.0	0.0	50.0	71.4	100.0	100.0	100.0	0.0	0.0	43.8	57.1
AW10-C	66.7	100.0	85.7	100.0	28.6	0.0	50.0	100.0	100.0	100.0	59.4	71.4
AW14-A	100.0	100.0	100.0	100.0	71.4	100.0	100.0	66.7	100.0	100.0	93.8	92.9
AW14-B	33.3	0.0	57.1	0.0	14.3	0.0	0.0	0.0	100.0	0.0	18.8	21.4
AW14-C	77.8	100.0	57.1	100.0	42.9	100.0	75.0	100.0	0.0	100.0	75.0	78.6
AX16-A	100.0	50.0	85.7	83.3	83.3	100.0	75.0	100.0	100.0	100.0	87.1	92.9
AX16-B	11.1	100.0	85.7	100.0	57.1	16.7	75.0	100.0	0.0	0.0	53.1	64.3
BC226-A	44.4	100.0	71.4	66.7	50.0	100.0	100.0	66.7	100.0	100.0	71.0	71.4
BC226-B	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	92.9
BC231-A	100.0	100.0	100.0	100.0	57.1	33.3	100.0	100.0	100.0	100.0	78.1	100.0
BC252-A	100.0	100.0	42.9	100.0	57.1	100.0	100.0	100.0	100.0	100.0	77.4	100.0
BC252-B	100.0	100.0	100.0	83.3	83.3	100.0	100.0	100.0	100.0	100.0	93.3	100.0

	Inodorus		Cantaloupensis									
Primer	Casaba	Honeydew	Charentais	European *Shipper*	Galia	Ogen	U.S. Eastern Market	U.S. Western Shipper	Conomon	Flexuosus	Europe	USA
BC280-A	100.0	100.0	85.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.9	100.0
BC280-B	100.0	100.0	100.0	100.0	85.7	100.0	100.0	100.0	100.0	0.0	96.9	92.9
BC299-A	44.4	0.0	14.3	66.7	100.0	100.0	100.0	66.7	0.0	0.0	61.3	57.1
BC318-A	100.0	100.0	100.0	100.0	85.7	100.0	100.0	66.7	0.0	100.0	96.9	85.7
BC318-B	66.7	0.0	14.3	0.0	71.4	100.0	0.0	0.0	100.0	0.0	56.3	7.1
BC388-A	77.8	100.0	14.3	100.0	71.4	100.0	75.0	100.0	100.0	100.0	71.9	85.7
BC388-B	0.0	100.0	28.6	100.0	28.6	0.0	100.0	100.0	100.0	0.0	34.4	64.3
BC403-A	100.0	100.0	100.0	100.0	85.7	100.0	100.0	100.0	100.0	100.0	96.9	100.0
BC403-B	100.0	100.0	100.0	100.0	85.7	100.0	100.0	100.0	100.0	100.0	96.9	100.0
BC407-A	100.0	100.0	100.0	100.0	100.0	83.3	100.0	100.0	100.0	100.0	96.8	100.0
BC407-B	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	92.9
BC407-C	0.0	0.0	14.3	0.0	0.0	0.0	0.0	33.3	100.0	0.0	3.2	14.3
BC469-A	66.7	0.0	42.9	83.3	57.1	100.0	75.0	100.0	0.0	0.0	62.5	71.4
BC469-B	100.0	100.0	28.6	100.0	57.1	100.0	75.0	100.0	0.0	100.0	78.1	78.6
BC469-C	22.2	0.0	42.9	0.0	14.3	0.0	50.0	0.0	100.0	0.0	12.5	35.7
BC526-A	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	92.9
BC526-B	66.7	0.0	14.3	16.7	57.1	83.3	75.0	33.3	0.0	100.0	40.6	64.3
BC526-C	55.6	100.0	85.7	100.0	42.9	33.3	50.0	66.7	0.0	100.0	62.5	64.3
BC551-A	88.9	100.0	100.0	100.0	85.7	100.0	100.0	100.0	100.0	100.0	93.8	100.0
BC551-B	100.0	50.0	57.1	100.0	42.9	0.0	75.0	100.0	100.0	100.0	59.4	85.7
BC605-A	11.1	50.0	14.3	33.3	42.9	0.0	0.0	0.0	0.0	0.0	18.8	14.3
BC617-A	100.0	100.0	100.0	100.0	80.0	100.0	100.0	100.0	100.0	100.0	100.0	92.9
BC627-A	11.1	0.0	0.0	50.0	14.3	0.0	25.0	100.0	100.0	0.0	12.5	42.9
BC628-A	88.9	50.0	71.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	90.6	92.9
BC628-A	11.1	100.0	42.9	16.7	14.3	100.0	75.0	0.0	0.0	100.0	37.5	42.9
BC628-B	44.4	50.0	85.7	100.0	42.9	100.0	25.0	100.0	100.0	100.0	71.9	64.3
BC642-A	100.0	100.0	85.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.8	100.0
BC642-A	44.4	100.0	42.9	66.7	71.4	83.3	100.0	66.7	0.0	100.0	59.4	78.6
BC642-B	44.4	100.0	42.9	66.7	33.3	100.0	100.0	66.7	0.0	100.0	61.3	64.3
BC646-A	44.4	50.0	42.9	33.3	66.7	50.0	100.0	0.0	0.0	0.0	41.9	57.1
BC646-A	100.0	100.0	100.0	100.0	83.3	100.0	100.0	100.0	0.0	100.0	96.8	92.9
BC652-A	100.0	100.0	28.6	100.0	85.7	100.0	100.0	66.7	100.0	100.0	84.4	85.7
BC652-B	44.4	100.0	14.3	100.0	71.4	100.0	100.0	66.7	0.0	100.0	65.6	71.4
BC654-A	88.9	50.0	57.1	66.7	42.9	100.0	0.0	66.7	0.0	100.0	71.9	42.9
BC654-B	88.9	50.0	71.4	0.0	42.9	0.0	25.0	0.0	0.0	0.0	40.6	35.7
BC663	100.0	50.0	100.0	100.0	100.0	100.0	100.0	66.7	100.0	100.0	96.9	92.9
Average	70.0	65.7	65.4	71.3	61.6	66.5	73.9	68.9	52.5	74.6	66.6	69.7
Std. dev.	33.5	40.1	34.6	38.3	30.2	43.1	36.6	38.2	50.1	43.7	28.1	26.3

[z]Band labeled as primer designation is followed by a upper case letter.

This primer set was chosen by Staub et al. (5) from a survey of 1,500 primers for its ability to detect polymorphism in an array of diverse melon germplasm. These primers are likely to maximize the detection of genetic variation in other arrays of melon germplasm. Information given herein allows strategic primer selection for development of RAPD marker test arrays in melon.

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Frequency of RAPD Polymorphisms in Melon (*Cucumis melo* L.) Germplasm in Different Geographic Regions

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Introduction: The genetic diversity of melon groups has been characterized using molecular analyses (5, 6, & 8). Random amplified polymorphic DNA markers have been used by García et al. (1) and Staub et al. (7) to assess the genetic diversity of elite germplasm. Likewise, Mliki et al. (3) and Nakata (4) used the same markers employed by Staub et al. (7) to define the diversity among African and Japanese germplasm, respectively. Mliki et al. (3) determined genetic differences among African landraces and between these landraces and elite U.S./European melon market class accessions used by Staub et al. (7). Nakata et al. (3) assessed the genetic variation of major Japanese melon market classes (i.e., House, Earl's and Oriental types), and then defined the genetic relationships between these market classes and a standard germplasm reference array (RA) drawn from accessions (Groups Cantaloupensis, Conomon, Inodorus, and Flexuosus) used by Staub et al. (7) and African landraces studied by Mliki et al. (3). We summarized herein the percentage of RAPD band presence within distinctly different geographic regions.

Materials and Methods: The RADP profiling of melon market classes by Staub et al. (7) included Charentais (7), European (6) and U.S. Western Shipper (3), U.S. Eastern Market (4), Galia (7), Ogen (6), Honeydew (2), Casaba (9), group Conomon (1), and group Flexuosus (1) accessions. These accessions were received from seed companies (5) and the U.S. Department of Agriculture, Agricultural Research Service. Mliki et al. (3) examined 108 exotic melon (*Cucumis melo* L.) accessions, and Nakata et al. (4) used 67 melon (*C. melo* L.) cultivars from five Japanese seed companies [Sakata (59), Yokohama Ueki (2), Nihon Engei Kenkyuukai (1), Kobayashi (4), and Tohoku (1)].

Molecular data were estimated from amplification products obtained by using 57 RAPD primers (Operon and University of British Columbia (BC)] (Table 1). A marker was considered repeatable if PCR yielded a consistent result in all of three (or more) replications (putative loci; see companion paper this issue). Tabulations summarize the percentage of RAPD band presence within a market class or subspecies and among European and U.S. germplasm as an estimation of the polymorphism level and diversity within groups. This was calculated as number of accessions with band presence divided by the total number of accessions examined and then multiplied by 100. This calculation is hereafter referred as percent frequency. When more than one polymorphic product per primer was obtained, the average of percent frequency of all the markers was tabulated. Accessions from the European, U.S., and African germplasm were further selected and used as reference accessions (RA) by Nakata et al. (4).

Results and Discussion: Germplasm examined from Europe, USA, and Japan should be considered elite since it presents either refined inbred lines or commercial hybrids. African landrace accessions are Group Conomon-like by their genetic relationship to the other germplasm as determined by RAPD marker analysis (4).

All primers used herein produced amplifications products that were polymorphic between and among groups (Table 1). Because different accessions were used in some analyses, the most appropriate comparisons are grouped in Table 1.

The majority of RAPD bands produced by any one primer were in a similar percentage in European and U.S. accessions. Notable exceptions were recorded in the comparison of European and U.S. germplasm using B12, AD12, AM2, BC318, BC388, BC551, BC627, and BC654. Relative percent frequency differences between these groups and corresponding RA groups were similar for most of the primers used. In rare cases the average percent frequency increased or decreased slightly when only portions of the accessions were accessed. This was the case when RA accessions were examined using AJ18, BC526, and BC551 in European and U.S. accessions, and when using C1, D7, AT5, and AW10 to examine

Table 1. Percentage of RAPD band presence detected in accessions from different geographic regions.

Primer	Europe[wx]	USA[w]	Africa[x]	Europe-RA[y]	USA-RA[y]	Africa-RA[y]	Japan[z]
B12	56.3	75.0	33.0				51.2
C1	93.8	85.7	97.0	63.5	54.2	46.4	67.5
D7	71.1	76.8	21.0	71.8	94.4	81.8	56.2
F1	96.9	92.9					
F4	87.5	92.9					
G8	46.9	42.9					
I4	62.5	89.3	26.0	61.5	83.3	40.0	71.6
I16	51.6	60.7	30.0	74.4	73.3	42.2	55.7
L18	55.1	47.6	36.5	73.1	58.3	43.3	52.2
N6	61.5	64.3					
W7	46.9	60.7	83.0			80.0	52.2
AB14	65.6	50.0					
AD12	71.9	85.7					
AD14	40.6	28.6	43.3			37.8	76.6
AE6	39.1	50.0					
AF7	76.0	69.0					
AF14	71.9	71.4	49.7	59.0	62.2	66.7	69.0
AG15	64.1	67.9	51.5	60.0	55.6	37.8	66.7
AJ18	67.2	78.6	38.0	19.2	16.7	43.3	34.3
AK16	96.9	85.7	43.0	97.4	88.9	42.2	91.0
AL5	37.5	42.9					
AM2	65.6	85.7					
AN5	68.8	73.8					
AO8	43.8	50.0					
AO19	82.8	85.7					
AS14	67.2	66.1					
AT1	71.9	78.6	66.7	61.5	61.1	62.2	59.4
AT2	77.1	69.0					
AT5	67.2	71.4	0.0	61.5	75.0	20.0	79.1
AT7	75.0	71.4					
AT15	68.8	71.4	51.0	96.2	91.7	43.3	84.3
AU2	30.2	42.9	56.5	53.8	55.6	55.6	41.5
AV11	76.6	67.9					
AW10	65.6	73.8	0.0	57.7	50.0	50.0	58.2
AW14	62.5	64.3	10.0	7.7	50.0	20.0	26.9
AX16	70.1	78.6	71.0	48.7	55.6	75.6	64.7
BC226	85.5	82.1					
BC231	78.1	100.0	32.5	83.3	40.0	66.7	55.2
BC252	85.4	100.0					
BC280	96.9	96.4					
BC299	61.3	57.1	60.0	61.5	40.0	80.0	17.9
BC318	76.6	46.4	90.0	100.0	100.0	73.3	87.1
BC388	53.1	75.0	62.0	76.9	83.3	73.3	44.8
BC403	96.9	100.0					
BC407	66.7	69.0					
BC469	51.0	61.9					
BC526	67.7	73.8	57.0	0.0	0.0	58.3	44.8

BC551	76.6	92.9	38.5	20.5	38.9	42.2	37.8
BC605	18.8	14.3					
BC617	100.0	92.9					
BC627	12.5	42.9					
BC628	66.7	66.7					
BC642	72.5	81.0					
BC646	69.4	75.0					
BC652	75.0	78.6					
BC654	56.3	39.3					
BC663	96.9	92.9					

[w]Data taken from Staub et al., (2000) (elite germplasm).
[x]Data of entire study taken from Mliki et al., (2001) (landraces).
[y]Data of standard accessions selected to from an array that defined the genetic diversity present in that data set (reference accessions, RA).
[z]Data taken from Nakata et al., (2001) (elite germplasm).

African RA accessions (Table 1). In other cases, change in the relative difference between RA groups was evident when AW14 and CBC231 were used to describe European and US accessions.

Some primers provided products at relatively low frequency in African germplasm that were evident in elite RA germplasm (e.g., AK16, AT5, AT15, and AW14) (Table 1). Also a lower percent frequency of band presence was observed in Japanese germplasm when compared to European, U.S., and African RA groups (e.g., D7, W7, BC299, and BC388) (Table 1).

This primer set was chosen by Staub et al. (5) for its ability to detect polymorphism in a broad array of melon germplasm. Thus, they are likely to maximize the detection of genetic variation in other arrays of melon germplasm.

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Powdery Mildew: An Emerging Disease of Watermelon in the United States

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Foliar diseases are common on watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai). Historically, anthracnose, gummy stem blight, and downy mildew have been the predominant foliar diseases encountered by U.S. growers (14). However, in the last few years, powdery mildew has emerged as an important disease problem of watermelon in the major U.S. production areas.

Powdery mildew symptoms on cucurbits typically appear as white powdery spots of mycelia and conidia on both sides of the leaves, but may appear on petioles and stems. Symptoms first develop on older leaves reducing plant canopy, and subsequent yield through decreased fruit size and number of fruit per plant (8). The reduced canopy may result in sunscald of the fruits making them unmarketable. The presence of the pathogen is much more readily apparent on pumpkin and squash than in watermelon, which can obscure visual detection until after plants are severely damaged by the disease. For example, watermelon leaves often begin deteriorating prior to obvious non-microscopic detection of mycelia and conidia, which makes early diagnosis and control on watermelon more difficult.

There are at least two different types of symptoms on watermelon. One is a yellow blotching (chlorotic spots) that occurs on leaves accompanied by little or no sporulation and only a small amount of mycelial development. The other symptom is powdery mycelial and conidial development on either leaf surface without the associated chlorotic spots. Often, the disease first appears as a slight yellowing of leaves in low areas of the field associated with higher relative humidity. However, an entire field should be scouted for early powdery mildew detection, since low areas are not always the first areas affected.

Because of the short disease cycle, control measures must be implemented soon after disease onset to be effective. Consequently, an accurate and rapid detection system is needed for effective powdery mildew control in watermelon. Research efforts on such a detection system are underway at the USDA, ARS South Central Agricultural Research Lab.

Two genera are considered the predominant fungi that incite powdery mildew in cucurbits, *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci and *Erysiphe cichoracearum* DC. *S. fuliginea*, has as many as seven pathogenically distinct races that are presently distinguished based on differential reactions against melon genotypes (9). Identity of the causal organism is important because *E. cichoracearum* and *S. fuliginea* differ in virulence against cucurbit species and in sensitivity to fungicides (3, 6, 7). Historically, powdery mildew has been rare on watermelon in the U.S. (11). Until recently, there was little incentive to study powdery mildew resistance in watermelon. However, in the last few years, powdery mildew has caused moderate to severe damage to watermelon crops in South Carolina, Georgia, Florida, Oklahoma, Texas, and California.

Methods. On 18 April 2000, 6-week-old seedlings of 111 *C. lanatus* entries were transplanted from the greenhouse to a field plot at Lane, Oklahoma for evaluation of powdery mildew resistance. Included in this study were two commercial cultivars, 102 plant introduction (PI) accessions from the USDA, ARS germplasm collection, Griffin, Georgia, one proprietary line, and six experimental hybrids (Table 1). Ten plants of each *C. lanatus* entry were planted in the non-replicated study.

On 7 June 2000, powdery mildew severity was assessed by rating the percentage of canopy damaged by the disease on a 1 to 5 scale where: 1 = 0% to 19% of a plant canopy affected by disease, 2 = 20% to 39%, 3 = 40% to 59%, 4 = 60% to 79%, and 5 = 80% to 100% of canopy damaged. Random field samples were taken to verify that powdery mildew was the only foliar disease present. The average rating for the 10 plants of each entry was plotted as a mean disease severity rating (Fig. 1).

Table 1. Powdery mildew rating for 111 watermelon cultivars, PI accessions and experimental lines.[z]

Rank	Cultivar or accession	Country of origin	Disease rating
1	PI 525088	Egypt	1.50
2	PI 482277	Zimbabwe	1.88
3	[1]L 200004	United States	2.00
4	[1]L 200006	United States	2.00
5	PI 225557	Zimbabwe	2.00
6	PI 273480	Ethiopia	2.00
7	PI 378611	Zaire	2.00
8	PI 459074	Botswana	2.22
9	PI 271776	South Africa	2.29
10	PI 482248	Zimbabwe	2.33
11	[1]L 200002	United States	2.50
12	PI 270545	Sudan	2.50
13	PI 500331	Zambia	2.67
14	[1]L 200003	United States	2.75
15	PI 249008	Nigeria	2.75
16	PI 186490	Nigeria	2.80
17	PI 482291	Zimbabwe	2.80
18	PI 254624	Sudan	2.83
19	PI 260733	Sudan	2.83
20	PI 274034	South Africa	3.00
21	PI 385964	Kenya	3.00
22	PI 542120	Botswana	3.00
23	[1]L 200005	United States	3.10
24	PI 542617	Algeria	3.14
25	[2]EXP 2000 PM	United States	3.17
26	PI 296341	South Africa	3.25
27	PI 490382	Mali	3.25
28	PI 254623	Sudan	3.29
29	PI 195928	Ethiopia	3.33
30	PI 254735	Senegal	3.33
31	PI 459075	Botswana	3.38
32	PI 270546	Ghana	3.40
33	PI 542116	Botswana	3.40
34	PI 482253	Zimbabwe	3.43
35	PI 500327	Zambia	3.44
36	PI 193964	Ethiopia	3.50
37	[1]L 200001	United States	3.57
38	PI 482247	Zimbabwe	3.60
39	PI 542114	Botswana	3.60
40	PI 246559	Senegal	3.67

Table 1 (continued).[z]

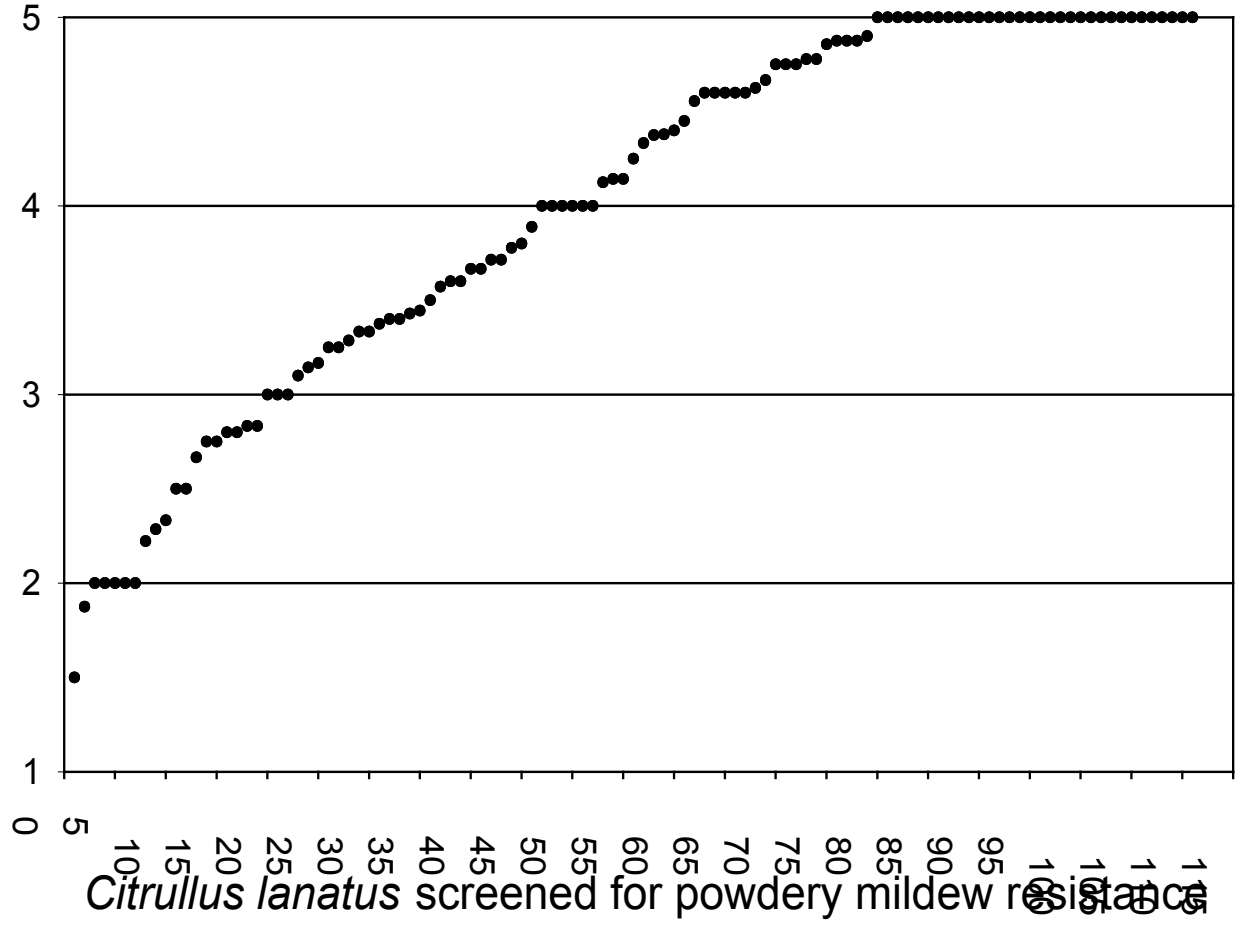
Rank	Cultivar or accession	Country of origin	Disease rating
41	PI 500336	Zambia	3.67
42	PI 482275	Zimbabwe	3.71
43	PI 490380	Mali	3.71
44	PI 270565	South Africa	3.78
45	PI 185635	Ghana	3.80
46	PI 295845	South Africa	3.89
47	PI 171392	South Africa	4.00
48	PI 183218	Egypt	4.00
49	PI 248178	Zaire	4.00
50	PI 481871	Sudan	4.00
51	PI 482284	Zimbabwe	4.00
52	PI 525096	Egypt	4.00
53	PI 254744	Senegal	4.13
54	PI 254622	Sudan	4.14
55	PI 494527	Nigeria	4.14
56	PI 490378	Mali	4.25
57	PI 271986	Somalia	4.33
58	PI 525084	Egypt	4.38
59	Tri-X 313	United States	4.38
60	PI 164247	Liberia	4.40
61	Royal Sweet	United States	4.45
62	PI 542121	Botswana	4.56
63	PI 186974	Ghana	4.60
64	PI 186975	Ghana	4.60
65	PI 271984	Somalia	4.60
66	PI 288232	Egypt	4.60
67	PI 525085	Egypt	4.60
68	PI 490381	Mali	4.63
69	PI 255139	South Africa	4.67
70	PI 189316	Nigeria	4.75
71	PI 254736	Senegal	4.75
72	PI 490375	Mali	4.75
73	PI 254738	Senegal	4.78
74	PI 254739	Senegal	4.78
75	PI 549163	Chad	4.86
76	PI 270547	Ghana	4.88
77	PI 494816	Zambia	4.88
78	PI 560000	Nigeria	4.88
79	PI 494815	Zambia	4.90
80	PI 164248	Liberia	5.00

Table 1 (continued).[z]

Rank	Cultivar or accession	Country of origin	Disease rating
81	PI 183217	Egypt	5.00
82	PI 184800	Nigeria	5.00
83	PI 185636	Ghana	5.00
84	PI 186489	Nigeria	5.00
85	PI 189317	Zaire	5.00
86	PI 193490	Ethiopia	5.00
87	PI 193963	Ethiopia	5.00
88	PI 195562	Ethiopia	5.00
89	PI 222137	Algeria	5.00
90	PI 254737	Senegal	5.00
91	PI 254741	Senegal	5.00
92	PI 271774	South Africa	5.00
93	PI 271982	Somalia	5.00
94	PI 271983	Somalia	5.00
95	PI 271987	Somalia	5.00
96	PI 273479	Ethiopia	5.00
97	PI 299563	South Africa	5.00
98	PI 306367	Angola	5.00
99	PI 319212	Egypt	5.00
100	PI 378615	Zaire	5.00
101	PI 392291	Kenya	5.00
102	PI 482260	Zimbabwe	5.00
103	PI 482269	Zimbabwe	5.00
104	PI 490377	Mali	5.00
105	PI 490384	Mali	5.00
106	PI 494821	Zambia	5.00
107	PI 500305	Zambia	5.00
108	PI 500320	Zambia	5.00
109	PI 525095	Egypt	5.00
110	PI 542115	Botswana	5.00
111	PI 559992	Nigeria	5.00

[z] The rating system consisted of 1 = 0% to 19% of a plant canopy affected by powdery mildew, 2 = 20% to 39%, 3 = 40% to 59%, 4 = 60% to 79%, and 5 = 80% to 100% of canopy affected; 1 indicates ARS, Lane, Oklahoma crosses; 2 indicates proprietary line from Syngenta Seeds.

Figure 1. Disease severity rating of 111 *C. lanatus* entries screened for powdery mildew resistance. Each dot represents the average disease rating for each of the 111 *C. lanatus* screened. The disease severity units are given on the left, which is an average of plant canopy ratings for each selection evaluated. The rating system consisted of 1 = 0% to 19% of a plant canopy affected by powdery mildew, 2 = 20% to 39%, 3 = 40% to 59%, 4 = 60% to 79%, and 5 = 80% to 100% of canopy affected.



Results. The powdery mildew pathogen present in the plots was identified as *S. fuliginea* (J.P. Damicone, Oklahoma State University), although the race was not determined. Disease severity ratings for the 111 *C. lanatus* entries ranged from 1.5 to 5.0 (Fig. 1). None of the entries exhibited immunity to powdery mildew, and only seven of the PIs screened had a disease severity rating of less than 2.0. Selections with a disease rating of 3.0 or less were considered moderately resistant to the *S. fuliginea* race present since plants with a 2.5 or lower disease severity rating showed no noticeable reduction in fruit quality or quantity. The commercial cultivars 'Tri-X 313' and 'Royal Sweet' had disease severity ratings of 4.4 and 4.5, respectively. Disease severity ratings among the PI accessions ranged from 1.5 to 5.0, of which 90 had ratings above 3.0, 63 had ratings of 4.0 and above, and 32 had ratings of 5.0.

Since powdery mildew epiphytotics have not been a problem on watermelon in the U.S. until recently, and the source of the apparent new strain is unknown. Perhaps a more virulent strain of powdery mildew was introduced, a new strain evolved, or a previously existing strain has become more prevalent. A similar situation was reported in Israel, where powdery mildew has recently become a limiting factor in watermelon production (4). Interestingly, Brazil has had a problem growing American watermelon cultivars due to susceptibility to the local race(s) of *S. fuliginea* (10).

Understanding the inheritance of powdery mildew resistance in cucurbits is complicated by the difficulty in differentiating between the two genera, and the races of powdery mildew that attack cucurbits. Further complicating this issue is the rapidity with which the predominant races can shift (13). Alvarez et al. (1) stated that the present classification of physiological races was inadequate for *S. fuliginea* isolates from Spain. They noted that different isolates belonging to race 2, based on current differentials (9), exhibited different patterns of virulence on certain melon genotypes. In Israel, the situation is even more perplexing. In 1988, Cohen and Eyal (5) reported that *C. lanatus* cultivars were resistant to race 1 of *S. fuliginea* and susceptible to race 2. More recently, Cohen et al. (4) stated that *S. fuliginea* race 1 isolates collected from cucumber and melon were infective only on the hypocotyls of watermelon. On the other hand, race 2 isolates from cucumber or melon failed to initiate disease on watermelon. In the U.S., Robinson et al. (11) stated that only one of the 590 *C. lanatus* accessions they tested was susceptible to an undetermined race of powdery mildew. In contrast, 248 of the 250 *C. lanatus* and *C. colocynthis* accessions tested in Israel were susceptible (4). Robinson et al. (12) used a susceptible accession from Belize (PI 269677) to study the inheritance of powdery mildew susceptibility in watermelon. Using PI 269677 X 'Sugar Baby' F1, F2, and backcross generations, they noted that susceptibility was due to a single recessive gene. Over the last several years, Thomas (unpublished data) has examined some powdery mildew isolates from watermelon in the U.S. Based on the widely-accepted conidial characteristics described by Ballentyne in 1963 (2), all of these watermelon isolates were identified as *S. fuliginea*. Based on inoculation tests to the cantaloupe differentials established by Pitrat et al. (9) to identify races of *S. fuliginea*, all of the isolates were race 2. However, these race 2 isolates from watermelon were more aggressive on race 2 susceptible differential cultivars, such as PMR 45, than older race 2 isolates that have been maintained at the U.S. Vegetable Laboratory in Charleston, SC.

The present study demonstrated a continuum in disease severity ratings from 1.5 to 5.0 among the watermelon entries screened. These preliminary data suggest that genetic resistance to the unknown race of *S. fuliginea* may be controlled by multiple genes. Since we are likely screening for resistance to a different race or strain of *S. fuliginea* compared to the 1975 studies (12), it is likely that a different array of genes may be involved in conferring the resistance observed.

Without resistant cultivars and with limited fungicide availability, U.S. watermelon growers could experience severe crop losses in years with optimal conditions for powdery mildew development. Currently, fungicide application and planting resistant cultivars remain the best controls for powdery mildew outbreaks on cucurbits. However, there is no published information available on relative resistance or susceptibility in U.S. watermelon cultivars against the new strain of *S. fuliginea*. Because of the capacity of this fungus to develop resistance to fungicides (7), alternating fungicides with different modes of action should be integrated into the disease management program.

In the present study *C. lanatus* entries were evaluated for resistance to a naturally-occurring, but as of yet undetermined strain of *S. fuliginea*. Based on this study some watermelon accessions are resistant to this undetermined, but highly virulent, strain of the pathogen. Crosses between PIs with low disease severity ratings, and commercial open-pollinated cultivars of *C. lanatus* have been produced to study the inheritance of this resistance.

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Use of an Insulin Syringe for Fusarium Wilt Inoculation of Watermelon Germplasm

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Sources of resistance to Fusarium wilt (*Fusarium oxysporum* Schlecht. (emend. Snyd. & Hans.) f. sp. *niveum* (E.F. Sm.)) in watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) has been an ongoing endeavor of many breeding programs. Several sources of resistance have been found, but this has generally been to races 0 and 1 (1, 4, 5). Netzer and Martyn (6) have identified a source of resistance to race 2, but it has not been generally incorporated into commercial sources. In addition, Fusarium wilt resistance can break down in the presence of root-knot nematode, which can further complicate breeding for resistance (7).

In 1998, we received a grant from the U.S. Dept. Agriculture to screen the available USDA watermelon germplasm collection for resistance to both Fusarium wilt race 2 and root-knot nematode ((*Meloidogyne incognita* (Kofoid & White) Chitwood). At the time the grant was awarded, there were approximately 1400 available accessions in the collection. This presented the problem of having reasonably reproducible inoculation techniques for Fusarium wilt and root-knot nematode.

The Fusarium wilt inoculation proved to be troublesome. Initially, we planned on using the tray dip method used by Martyn and Netzer (5). Immediately, it was recognized that this would be impossible with our limited resources. As the trays are held in the inoculum, the trays absorb the solution so that very quickly the inoculum is used up. We lacked the facilities to produce the large volumes of inoculum the technique would require.

The plant inoculum technique (5) was also rejected because of the difficulty of removing plants from the soil, inoculating, and replanting. Finally, the infested soil technique could not be used because Fusarium wilt race 2 has not been isolated in Georgia and we were prohibited from inoculating under field conditions.

This study was undertaken to determine if a small quantity of inoculum could be injected with a syringe into each plant with acceptable development of disease.

Methods. Plants were seeded in the greenhouse into 28 x 56 cm flats with 809 inserts (8 packs of 9 cells, 3.8x3.8x6.4 cm) filled with Metromix 300 soil mix (Scotts-Sierra Products Co., Marysville, OH). The experiment design was a 5 x 4 factorial arranged in a randomized complete block with 8 replications and 9 plants per treatment combination. Plants were seeded on 2 March 1998 and inoculated 2 weeks later. Plants were evaluated 4 weeks after inoculation on a 0 to 9 scale (0=no signs of disease progressing, 9=death of the plant).

The inoculum was prepared with potato dextrose broth (Fisher Scientific, Norcross, GA) according to manufacture's directions. This broth was inoculated with Fusarium wilt race 2, which was obtained from Dr. Fenny Dane (Auburn University). This was the same culture as stored with American Type Culture Collection (Manassas, VA), culture number 62939. The inoculated broth was kept agitated at 20 C for 2 weeks. At this time, the inoculum was adjusted to $1.5x10^6$ microspores per ml with a hemacytometer.

Five inoculation treatments were used: a control (nothing), insulin syringe with 50 µl of water, insulin syringe with 50 µl of inoculum, tray dip in water for 10 minutes, tray dip in inoculum for 10 minutes (5). The insulin syringe (Becton Dickinson Co., Franklin

Lakes, NJ) was a 0.5 cc (50 unit) syringe graduated in 50 µl (5 unit) increments. Each plant inoculated with a syringe had 50 µl of solution injected at the base of the stem just above the soil line. Plants were not placed back into the flats after inoculation to prevent contamination of adjacent packs.

The second factor tested was cultivar. The four cultivars tested were AU-Producer (Hollar Seed Co., Rocky Ford, CO), Starbrite (Asgrow Seed Co., Gonzales, CA), PI 296341-FR, and a self-pollinated selection of PI 296341-FR. AU-Producer is an open-pollinated, Fusarium wilt race 2 susceptible cultivar. Starbrite is an F_1 hybrid susceptible cultivar. PIs 296341-FR and 296341-FR self are a resistant selection and its progeny, respectively.

Results. There were no differences among the cultivars tested and there was no interaction effect between cultivars and inoculation technique (Table 1). However, there were differences among the inoculation techniques. Untreated plants had the lowest rating of 1.1 and differed significantly from all other inoculation techniques. The insulin syringe with Fusarium wilt inoculum had a more severe incidence of Fusarium wilt compared to the syringe with water. However, both of these techniques had lower disease severity ratings compared to the tray dip with Fusarium wilt or tray dip with water (Table 1).

The lack of differences among the cultivars is surprising but not unexpected. Fusarium wilt reaction among resistant and susceptible cultivars is highly variable and under some conditions can cause soil suppression of the pathogen (2). This high degree of variability of results may also be due to mutability of the pathogen. Different reactions even under the most stringent controls can be particularly troublesome. Interactions between cultivars, races of the pathogen, and soil microbes are apparent but not completely understood (3).

Table 1. Cultivar and inoculation technique effects on mean rating for Fusarium wilt race 2 reaction in watermelon.

Treatment	Rating[z]
Cultivar	
AU-Producer (susceptible)	3.6a
Starbrite (susceptible)	3.5a
PI 296341-FR (resistant)	4.3a
PI 296341-FR Self (resistant)	3.7a
Inoculation Technique	
Nothing	1.1c[y]
Syringe with water	2.4c
Syringe with Fusarium	3.4b
Tray dip with water	5.6a
Tray dip with Fusarium	6.3a
Probability > F	
Cultivar	0.418
Inoculation Technique	0.000
Cultivar x Inoculation Technique	0.974

[z]0-9 rating scale: 0-no disease present, 9-severe disease symptoms.
[y]Means followed by the same letter in a column are not different by Fisher's Protected LSD ($p \geq 0.05$).

The fact that there was no difference between the tray dip with Fusarium wilt and the tray dip with water is also not completely unexpected. Similar observations have been seen with uninoculated watermelon plants placed in high humidity/high temperature incubation chambers where these plants have reacted in a similar fashion to those that have been inoculated with a foliar pathogen prior to placement in the incubation chamber.

Although the syringe with Fusarium wilt inoculum had a lower disease reaction compared to the tray dip with or without inoculum, it was better than the syringe with water alone. Based on these results and the logistics of having to deal with such a large collection with a limited supply of inoculum, it was decided the syringe method would be better for our needs.

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Characterization of a New Male Sterile Mutant in Watermelon

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Watermelon, *Citrullus lanatus* (Thunb.) Matsum. and Nakai, is an important crop worldwide. Hybrid cultivars are replacing open pollinated cultivars because hybrids are thought to have higher yield and quality, and more uniform fruit size. However, the cost of hybrid seed is much higher than that of open-pollinated seed because of extra labor required for controlled pollination in producing hybrid seed. Male sterility has been found in many crop plants, and has been used to reduce the costs of hybrid seed production (Kaul, 1988).

The first male sterility in watermelon was reported by Watts (1962) who found a male sterile mutant in the X2 generation of 'Sugar Baby' irradiated with gamma rays. The mutant was described as a glabrous male sterile (*gms*) due to the associated lack of hairs on the plant foliage (Watts, 1962). Glabrousness and male sterility were inherited together as a single recessive nuclear gene, suggesting very close linkage or a pleiotropic effect of the locus involved (Watts, 1967). The *gms* gene not only disrupts the male reproductive function, but also reduces female reproduction (Watts, 1967). Therefore, there has been little commercial application of the *gms* gene (Zang et al., 1994).

A second male sterile mutant was reported by Zang et al. (1990). Two spontaneous male sterile mutants were found in a self-pollinated population of a commercial cultivar 'Nongmei 100' in China in 1983. These mutants were sib-mated and used to develop breeding line G17AB, which has desirable agronomic traits (Zang et al., 1990). In contrast to the *gms* trait, the Chinese male sterile line contains no gross morphological differences between sterile and fertile plants (Zang et al., 1990). Female fertility of the Chinese male sterile mutant is normal and there is stability for male-sterile and female-fertile characters despite varying environmental conditions (Zang et al., 1994). The Chinese male sterile mutation was inherited as a single recessive nuclear gene (Zang et al., 1990), and assigned the gene symbol, *ms* (or *ms-1* if additional loci were identified). The cytological phenomenon associated with male sterility in the Chinese male sterile line is the lack of the tapetum, and tetrads that do not form (Zang et al., 1994).

In the study presented here, we investigate the characteristics of a new male sterile mutant found in Korea.

Methods. A third occurrence of male sterility was observed as a mutant in a breeding line, unrelated to the glabrous male sterility and Chinese male sterility in 1998 in Korea. This line originated from a cross between the commercial hybrid cultivar 'Fiesta' (Syngenta) and a breeding line with high quality, HL229 (Hungnong Seed Co.).

A male sterile mutant was found in the progeny during self-pollination to develop inbred lines. Five plants in a population of twenty failed to set fruit from self-pollinated flowers. The anthers of the five plants were smaller than those of their sibs, and they usually failed to dehisce. Microscopic examination disclosed that they contained no viable pollen.

This investigation was initiated to study the genetics of the the new male sterile mutant. Five male sterile plants set fruits readily when pollinated by fertile sibs. Five progenies were obtained from the five male sterile plants after cross-pollination with five of their fifteen fertile sibs. In addition, self-pollinated progenies were obtained from fifteen fertile sibs. A check watermelon line, heterozygous for the Chinese male sterile gene, *ms*, was kindly provided by Dr. G. E. Tolla, Seminis Vegetable Seeds / Asgrow Brand, USA.

Results. Progeny tests demonstrated that eight of the fifteen fertile sibs, which produced all-fertile progenies, were homozygous normal (Table 1). Seven, which produced progenies segregating approximately three male fertile to one male sterile, were heterozygous for a single, recessive factor for male sterility. The progenies from two male sterile plants pollinated by male fertile sibs segregated one male fertile to one male sterile, and the progenies from three other sterile plants produced all fertile progeny after sib mating. A total population of 210 plants from self-pollinated heterozygous parents, in seven generations, segregated 160 normal and 50 male sterile plants (chi-square 3:1, 0.16). Similarly, 80 plants in two progenies from male sterile plants pollinated by heterozygous sibs segregated 43 normal and 37 male sterile plants (chi-square 1:1, 0.45).

In order to determine the nature of the genetic relationship between the Chinese male sterile and the new male sterile, a plant homozygous for the new male sterile factor was crossed with a plant heterozygous for the Chinese male sterile gene. The reciprocal cross was made in the spring of 1999. All of the 367 F1 plants grown in the greenhouse in the autumn of 1999 revealed no segregation for sterility in the genotypes, indicating that there were two loci involved, with fertile x sterile sibs having genotypes ms-1/ms-1 Ms-2/Ms-2 x Ms-1/Ms-1 Ms-2/ms-2 or Ms-1/Ms-1 ms-2/ms-2 x Ms-1/ms-1 Ms-2/Ms-2 (Table 2). The data indicated that the Chinese male sterile gene and the new male sterile were non-allelic. Murdock et al. (1990) reported that the Chinese male sterile gene, *ms*, and the glabrous male sterile gene, *gms*, were non-allelic. We propose the symbol *ms-2* for the new Korean male sterile, making it the second in the series of male sterile genes, after the Chinese male sterile, *ms* (or *ms-1* for clarity).

The cross of genotypes *ms-1/ms-1 Ms-2/Ms-2 x Ms-1/Ms-1 Ms-2/ms-2* produced all fertile F1 progeny. Thirteen of these F1 plants were self-pollinated in the autumn of 1999, and 50 F2 progeny from each were evaluated for the presence of *ms-1* and *ms-2* in spring of 2000. Half of the F2 progenies made by crossing with F1 hybrids heterozygous for only *ms-1* would be expected to segregate normal and male sterile plants at a 3:1 ratio. Seven of the F1 plants (termed P3, P4, P8, P9, P10, P11, and P13) segregated in this manner (Table 3). The 331 plants in the population included 251 normal and 80 male sterile plants. The observed numbers fit a 3:1 ratio (chi-square 0.12) and differed almost significantly from a 9:7 ratio (chi-square 51.6). Therefore, the progenies were determined to have the *Ms-1/ms-1 Ms-2/Ms-2* genotype. Half of the F2 progenies, from F1 hybrids heterozygous for both *ms-1 and ms-2* would be expected to segregate normal and male sterile plants in a ratio differing from the 3:1 ratio. If *ms-1 and ms-2* were independently inherited, the F1 progenies would be expected to segregate normal and male sterile plants in a 9:7 ratio. Six of the F1 plants (termed P1, P2, P5, P6, P7, and P12) segregated in this manner (Table 3). The 307 plants in the population included 179 normal and 128 male sterile plants. The observed numbers fit a 9:7 ratio (chi-square 0.53) and differed almost significantly from a 3:1 ratio (chi-square 45.6). Therefore, the progenies were determined to have the *Ms-1/ms-1 Ms-2/ms-2* genotype.

The new male sterile mutant was first observed in 5 of 20 plants in watermelon progeny 1255. That F7 progeny was derived from the cross between cultivar 'Fiesta' and breeding line HL229. Progeny tests demonstrated that the gene, *ms-2*, occurred in the 330-10 parent of 1255 (Fig. 1). The mutation probably occurred in one of the two germ cells from plant 1121-4 that produced 330-10 or as a somatic mutation in 330-10 itself. The first alternative could result from unequal crossing-over or another type of minute chromatin loss, gain, or qualitative change at meiosis. The second alternative could result from failure of minute chromatin duplication, loss of a minute chromatin fragment, or a qualitative change during meiosis.

Development of staminate flowers was affected by the new male sterile mutant. The size of the sterile staminate flower bud was same as that of fertile staminate flower until 3 to 4 days before anthesis. Thereafter, the size of the fertile staminate flower increased greatly in one day before anthesis, whereas the size of sterile staminate flower remained relatively small during its development (Fig. 2 C, D). The same phenomenon was reported in the Chinese male sterile line (Zang et al., 1994). The size of sterile staminate flower in the newly found male sterile mutant is bigger than that of the sterile staminate flower in the Chinese male sterile line one day before anthesis (Fig. 2 B, D). The development of anthers showed great difference between the fertile and sterile flowers (Fig. 3). The size of the fertile anther increased greatly in the day before anthesis, whereas the size of the sterile male anther remained

Table 1. Progeny test of male sterile plants and their male fertile sibs.

Progeny	Number of plants Total	mf	ms	Ratio Obs:Exp	Chi-square	Probability
ms(3) x mf(2)	40	22	18	1 : 1	0.4000	0.750>P>0.500
ms(10) x mf(12)	40	21	19	1 : 1	0.1000	P=0.750
ms(11) x mf(13)	40	40	0			
ms(15) x mf(16)	40	40	0			
ms(20) x mf(19)	40	40	0			
mf(2) selfed	30	23	7	3 : 1	0.0400	0.900>P>0.750
mf(5) selfed	30	24	6	3 : 1	0.4000	0.750>P>0.500
mf(8) selfed	30	23	7	3 : 1	0.0400	0.900>P>0.750
mf(9) selfed	30	22	8	3 : 1	0.0400	0.900>P>0.750
mf(12) selfed	30	21	9	3 : 1	0.4000	0.750>P>0.500
mf(17) selfed	30	24	6	3 : 1	0.4000	0.750>P>0.500
mf(18) selfed	30	23	7	3 : 1	0.0400	0.900>P>0.750
mf(1) selfed	30	30	0			
mf(4) selfed	30	30	0			
mf(6) selfed	30	30	0			
mf(7) selfed	30	30	0			
mf(13) selfed	30	30	0			
mf(14) selfed	30	30	0			
mf(16) selfed	30	30	0			
mf(19) selfed	30	30	0			

Table 2. Allelism test for male sterility genes between the Chinese male sterile line and the new male sterile line.[z]

Female parent	Male parent	Number of plants Total	mf	ms
Chinese ms plant (*ms-1/ms-1 Ms-2/Ms-2*)	New mf plant (*Ms-1/Ms-1 Ms-2/ms-2*)	178	178	0
New ms plant (*Ms-1/Ms-1 ms-2/ms-2*)	Chinese mf plant (*Ms-1/ms-1 Ms-2/Ms-2*)	189	189	0

[z] Proposed gene symbols for watermelon male sterile, designating *ms*-1 for the Chinese allele and *ms*-2 for the new Korean male sterile.

Table 3. Segregation of F2 progeny from sib mated F1 plants (*ms-1/ms-1 Ms-2/Ms-2 x Ms-1/Ms-1 Ms-2/ms-2*).

F2 progeny from F1 plants	Number of plants Total	mf	ms	Ratio Obs:Exp	Chi-square	Probability
P3	53	42	11	3:1	0.5094	0.500>P>0.250
P4	53	39	14	3:1	0.0566	0.900>P>0.750
P8	53	44	9	3:1	1.8176	0.250>P>0.100
P9	43	30	13	3:1	0.6279	0.500>P>0.250
P10	42	34	8	3:1	0.7937	0.500>P>0.250
P11	42	31	11	3:1	0.0317	0.900>P>0.750
P13	45	31	14	3:1	0.8963	0.500>P>0.250
Pooled	331	251	80	3:1	0.1219	0.750>P>0.500
P1	53	33	20	9:7	0.7790	0.500>P>0.250
P2	53	32	21	9:7	0.3669	0.750>P>0.500
P5	53	30	23	9:7	0.0027	P>0.950
P6	53	29	24	9:7	0.0506	0.900>P>0.750
P7	53	28	25	9:7	0.2519	0.750>P>0.500
P12	42	27	15	9:7	1.1020	0.500>P>0.250
Pooled	307	179	128	9:7	0.5274	0.500>P>0.250

Year	Season	Progeny generation	Diploid -plant	Genotype (ms)
1995	spring	F1	343-6	+/+
			sibs	+/+
1996	spring	F2	328-7	+/+
			sibs	+/+
1997	spring	F3	329-6	+/+
			sibs	+/+
1997	fall	F4	1121-4	+/+
			sibs	+/+
1998	spring	F5	330-10	+/*ms*-2
			sibs	+/+
1998	fall	F6	1255-1	+/+
			-2	+/*ms*-2
			-3	*ms*-2/*ms*-2
			-4	+/+
			-5	+/*ms*-2
			-6	+/+
			-7	+/+
			-8	+/*ms*-2
			-9	+/*ms*-2
			-10	*ms*-2/*ms*-2
			-11	*ms*-2/*ms*-2
			-12	+/*ms*-2
			-13	+/+
			-14	+/+
			-15	*ms*-2/*ms*-2
			-16	+/+
			-17	+/*ms*-2
			-18	+/*ms*-2
			-19	+/+
			-20	*ms*-2/*ms*-2

Figure 1. Probable origin of the recessive allele (*ms-2*) responsible for the new male sterility of watermelon found in 1998 in Korea.

Figure 2. Development of sterile and fertile staminate flower bud (from 1 to 7, staminate flower bud from the next node of bloomed staminate flower to the seventh node): A, Fertile of the Chinese male sterile line. B, Sterile of the Chinese male sterile line. C, Fertile of the new male sterile mutant. D, male sterile of the new male sterile mutant.

Figure 3. Development of male sterile and fertile anther (from 1 to 7, anther from the next node of bloomed staminate flower to the seventh node). A: male fertile of the Chinese male sterile line. B: male sterile of the Chinese male sterile line. C: male fertile of the new male sterile mutant. D: male sterile of the new male sterile mutant.

Figure 4. Comparative morphology of staminate flower between the newly found male sterile mutants and the Chinese male sterile line. Top left: sterile staminate flower of the Chinese male sterile line. Bottom left: fertile staminate flower of the Chinese male sterile line. Top right: sterile staminate flower of the newly found male sterile mutant. Bottom right: fertile staminate flower of the newly found male sterile mutant.

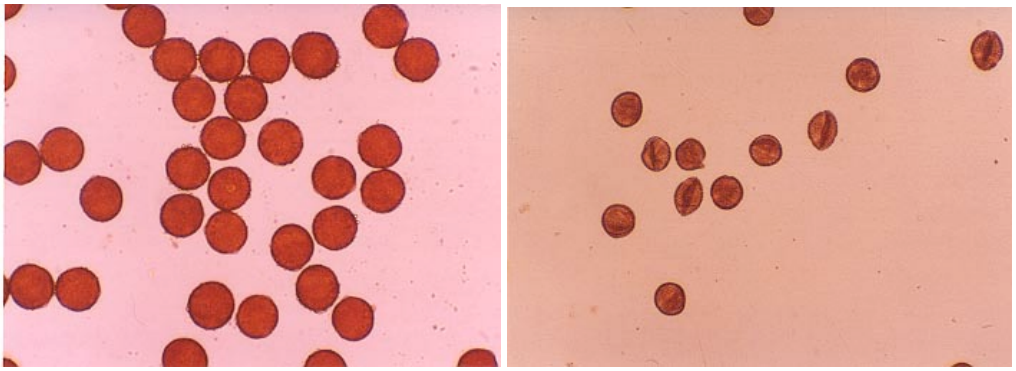

Figure 5. Specimens from the anthers of mature watermelon staminate flowers. Left: normal pollen from a male fertile sib (x100). Right: sterile pollen from male sterile plant of the new male sterile mutant (x100).

relatively small during its development (Fig. 3 C, D). The size of sterile anther in the new male sterile mutant is larger than that of sterile staminate flower in the Chinese male sterile line in the day before anthesis (Fig. 3 B, D). The buds from the male sterile plants became yellowish and almost fail to dehisce. The staminate flowers from the male sterile plants were open sometimes, but the size was smaller than that of the normal flower and no viable pollen was produced (Fig. 4 and 5).

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Germination of Watermelon Seeds at Low Temperature

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The Cucurbitaceae comprise more than 700 species of herbaceous crops plants with about 90 genera. They provide vegetables, fruits, oils, and several other useful products. The fruits are a pepo (fleshy berry-like structures with a rind and spongy seed interiors), but sometimes a papery, bladdery pod. The seeds are usually flat and plate-like.

Watermelon (*Citrullus lanatus* (Thumb.) Matsum. and Nakai) was formerly *Citrullus vulgaris*. Commercial cultivars are classified as *Citrullus lanatus* var. *lanatus*, and wild accessions are *Citrullus lanatus* var. *citroides*. It has been cultivated for thousands of years as indicated by the fact that it has a name in Sanskrit. Watermelon is grown throughout the world as a staple food (edible seeds), a dessert food (edible flesh), and for animal feed, although it is primarily eaten fresh. It is also eaten as a cooked vegetable in Africa. In Russia, watermelon is a staple food eaten pickled and used for production of syrup by boiling the sugary flesh. In China, firm-fleshed cultivars are cut into strips and dried for use as pickles or glace candy.

In the U.S., watermelon is a major vegetable crop that is grown primarily in the southern states. The major watermelon producing states in the U.S. are Florida, California, Texas, Georgia, and Arizona. Total area under production in the U.S. in 1998 was 76,629 ha, with a total production of 36,731 Mg (U.S.D.A., 1998).

Early-planted watermelons often have difficulty with seed germination and emergence. Cultivars selected for cold germination ability would provide growers with better stands for crop production.

The seeds of cucurbitaceous crops are non-endospermic and germination is epigeal. Dormancy can be a severe problem in some species. It is comparatively easy to induce dormancy by testing the seeds for germination in unfavorable environments. Ellis et al. (1985) provides specific germination information and test recommendations.

Poor field emergence and erratic stands lead to increased variation in plant development, which can result in yield reductions. The survival and performance of seeds after sowing is affected by physical, mechanical, chemical and biotic factors. Temperature, light, drought, flooding and gaseous environments are physical factors which influence seedling emergence (Khan et al., 1979; Hegarty, 1979; Thomas, 1981). Low temperature after the sowing of many warm-season vegetables can lead to asynchronous seedling emergence (Kotowski, 1962; Thompson, 1974). This asynchrony is consistently observed where spring temperatures fluctuate dramatically.

For crops harvested once-over by machine, non-uniform emergence is of particular concern. Cucurbit seeds require high temperatures for successful germination and seedling emergence (Harrington and Minges, 1954; Hegarty, 1973). For instance, cucumber (*Cucumis sativus* L.) seeds germinated rapidly at 20°C, but the time to 50% germination at 14°C decreased substantially (Simon et al., 1976; Nienhuis et al., 1983), and below 11°C only a small percentage of the seeds germinate (Simon et al., 1976). Germination of watermelon seeds (*Citrullus lanatus* (Thumb.) Matsum. and Nakai) was improved at 15°C after priming with inorganic osmotica (Sachs, 1977). Several treatments of cucumber seeds did not improve germination, however (Staub et al., 1989).

Nilsson (1968) reported that in cucumber there was genetic variation among cultivars for germination at 12, 14, 17 and 23°C, with 'Rhenskdruv', P5261 and 'Favor' best and 'Hokus' and 'Rea' worst. Seget (1966) reported that in two cucumber cultivars at soil temperature below 12°C, depression of emergence was caused mainly by the direct effect of low temperatures of 12 to 16°C on the plants. It was due to mainly to the effect of pathogenic and parasitic micro-organisms. With a prolongation of the low temperature effect, emergence was markedly reduced. Except in extreme cases, the soil moisture

content had no significant effect on emergence at low temperatures. Emergence was higher in sterilized than in unsterilized soils, and seed treatment was always beneficial.

Hall et al. (1989) reported that water-imbibed watermelon seeds provided faster crop establishment than dry seeds at temperatures below the optimum (15.7°C). Mozumder et al. (1970) reported that conidia were germinated at three temperatures in media in which available water, expressed as water activity, was controlled at three levels. Rate of germination in the basal medium (0.9964 water activity) was most rapid at 25°C and was inhibited at 15 and 30°C. Lowering the water activity at particular temperatures decreased the rate of germination. However, at a low water activity (0.9778) germination rate was greater at 30 than at 25°C. Thus the effect of temperature on germination appeared to be dependent on the water activity of the medium.

Milotay et al. (1991) reported that the germinating capacity of seeds of 15 cultivars and lines was studied in the laboratory at constant and fluctuating temperatures ranging from 15°C. Temperature affected both the time required to start germination and the initial growth of the seedlings. At 17°C, significant differences among cultivars were found for germination percentage and radicle growth, and starting date was more variable. Germinating capacity decreased drastically with temperature. At 17 to 25°C, a positive correlation was found between 1000-seed weight and germination percentage. Uniform germination could be expected above 17°C, and therefore early sowing in cold soil should be avoided. It was possible to measure the growth rate and calculate vigor by germinating seeds in vertically-placed filter paper rolls.

Jennings and Saltveit (1994) reported that cucumber seeds ('Dasher II' and 'Poinsett 76') were imbibed and germinated at 10 to 30°C, and seeds germinated at 25°C for 24h were chilled at 2.5 for 0 to 144h. Both cultivars responded similarly to the treatments in terms of fresh weight increases, time to radicle emergence and root growth. In comparison, seeds from an aged (1989) lot of 'Poinsett 76' responded very differently from the 1992 seed lots in all experiments. The chilling tolerance levels of germinating 'Poinsett 76' seeds varied with seedling age as measured by resumption of root growth. The results suggest that some factor that confers chilling tolerance is gradually lost during the early stages of germination following imbibition.

Roeggen (1987) reported that cultivars of bean, cucumber and tomato were tested with the aim of finding any differences in minimum germination temperature. A variation in minimum germination temperature from 7.0 to 10.5°C in tomato cultivars. Pesticide treatment of the seeds of the bean cultivar 'Processor' had a restrictive effect on germination.

The objectives of this study were to determine the optimum temperature for testing watermelon seeds for cold germination ability. In addition, we were interested in whether there were genetic differences among cultivars for cold germination ability.

Methods. Two experiments were run, the first to determine the optimum temperature to use for cold germination tests. Four temperatures were used, based on previous experience, and a review of the literature: 10, 14, 18, and 22°C (Table 1). The second experiment was to screen a diverse set of cultivars for germination speed at the chosen temperatures.

For the cultivar experiment, 44 diverse cultivars were chosen (Table 2). Seeds were treated with a fungicide (captan) to prevent surface organisms from rotting the seeds during the test. Germination data was recorded daily. Data were used to calculate percentage germination, actual days to germinate (for seeds that actually germinated), and total days to germinate (for all seeds). For total days to germinate, seeds that failed to germinate by the end of the test (approximately 30 days) were considered to have germinated on the last day.

Seeds were germinated in controlled temperature chambers in the Phytotron at North Carolina State University (Downs and Thomas, 1983). Temperatures were set at 10, 14, 18, or 22°C. Each treatment combination consisted of 10 seeds in one plastic Petri plate (100 mm diameter). Each plate had two pieces of filter paper wetted with 1.5 ml of water (equilibrated to the chamber temperature).

Seeds were placed in the chambers on 13 October (replications 1 to 4) or 7 November (replications 5 to 8) 2000 for the temperature experiment. Seeds were placed in the chambers on 30 October for the cultivar

Table 1. Germination percentage and speed of two watermelon cultivars tested at four temperatures in the growth chamber.[z]

Temperature (°C)	Cultivar name	Days to actual germination	Days to total germination	Percentage germination
10	Charleston Gray	-	-	0
	Petite Sweet	-	-	0
14	Charleston Gray	19.4	26.4	46
	Petite Sweet	19.3	26.8	44
18	Charleston Gray	11.7	15.4	83
	Petite Sweet	11.3	17.7	70
22	Charleston Gray	5.9	10.3	84
	Petite Sweet	8.4	13.6	79
LSD (5%)		*2.2*	*2.6*	*15*
CV (%)		*17*	*12*	*29*
Mean	Charleston Gray	12.3	17.4	71
	Petite Sweet	13.0	19.7	64

Correlation (% germ. vs. days to germ.) = -0.68
Correlation (days to actual vs. days to total) = 0.91

[z] Data are means of 2 seeding dates of 4 replications of 10 seeds each, tested for 32 days. Data for 10°C were excluded from the means since there was no germination. Seeds were considered germinated when the radicle emerged 10 mm out of the seed coat. Actual days to germinate was calculated for seeds that actually germinated. Total days to germinate was calculated for all seeds (seeds that failed to germinate by the end of the test were considered to have germinated on the last day).

Table 2. Germination percentage and speed of 44 watermelon cultivars tested at two temperatures in the growth chamber.[z]

	14°C chamber			18°C chamber		
Cultivar	Total	Actual	%	Total	Actual	%
Starbrite	10.8	8.2	90	7.0	5.6	95
Blackstone	14.2	12.9	95	5.6	5.6	100
Crimson Sweet	18.5	10.1	65	5.1	5.1	100
AU-Jubilant	19.1	14.2	75	10.4	6.4	85
Sultan	20.1	8.7	55	5.1	5.1	100
AU-Golden Producer	20.7	14.7	70	5.5	5.5	100
Jubilee	21.4	13.0	60	12.8	5.7	75
Jubilee II	23.7	18.6	70	8.9	6.1	90
Fiesta	24.5	11.0	40	4.4	4.4	100
Dixielee	24.5	10.0	40	9.6	8.5	95
Orangeglo	24.5	10.1	40	18.0	7.1	60
AU-Sweet Scarlet	24.7	13.2	45	7.5	7.5	100
Dixie Queen	24.9	18.8	60	10.2	7.7	90
Sugar Baby	25.8	10.5	35	5.5	5.5	100

Stars-N-Stripes	26.3	12.0	35	8.5	5.6	90
Klondike Striped #11	26.7	12.8	35	5.6	5.6	100
Super Sweet	27.2	11.4	30	7.6	6.2	95
Big Crimson	29.4	18.7	30	5.5	5.5	100
Minilee	30.5	10.8	15	5.6	5.6	100
Desert King	30.6	22.2	20	5.7	5.7	100
Petite Sweet	31.0	23.8	30	22.3	12.7	55
Graybelle	31.4	7.5	10	6.6	5.1	95
Calhoun Gray	31.4	28.4	35	9.6	9.6	100
Calsweet	31.5	28.1	40	5.7	5.7	100
Bush Jubilee	31.5	26.1	35	18.8	13.8	75
Peacock Shipper	31.7	18.3	15	5.6	5.6	100
Kleckley Sweet	32.5	18.5	10	11.4	5.7	80
Sugarlee	32.8	30.1	45	14.1	9.0	80
Regency	32.9	12.0	5	7.2	7.2	100
Peacock WR60	32.9	22.5	10	20.3	12.9	65
Long Crimson	33.0	14.0	5	9.2	6.5	90
Black Diamond YF	33.0	13.0	5	14.5	10.9	85
Sweet Princess	33.4	21.0	5	18.5	13.0	70
Louisiana Sweet	33.7	28.0	5	18.0	15.2	85
Super Gold	33.8	32.8	20	5.4	5.4	100
Tendergold	33.9	33.0	10	8.2	6.9	95
Chubby Gray	33.9	33.0	15	9.6	8.3	95
Black Diamond YB	34.0	33.0	5	5.3	5.3	100
Tastigold	34.0	33.0	5	20.8	10.0	55
Mickylee	-	-	0	5.3	5.3	100
AU-Producer	-	-	0	6.0	4.5	95
Golden Honey	-	-	0	16.8	9.5	65
Yellow Crimson	-	-	0	22.7	14.4	50
Verona	-	-	0	22.9	11.8	50
LSD both temp. (5%)				*4.1*	*3.6*	*8.1*
CV both temp. (%)				*20*	*28*	*31*

Correlation (% germ. vs. days to germ.) = -0.66**
Correlation (days to actual vs. days to total) = 0.79**
Correlation (days to actual at 14°C vs. days to actual at 18°C) = 0.39*
Correlation (days to total at 14°C vs. days to total at 18°C) = 0.40*
Correlation (% germ at 14°C vs. % germ at 18°C) = 0.41*

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[z] Data are means of 2 replications of 10 seeds each, tested for 32 days. Seeds were considered germinated when the radicle emerged 10 mm out of the seed coat. Actual days to germinate was calculated for seeds that actually germinated. Total days to germinate was calculated for all seeds (seeds that failed to germinate by the end of the test were considered to have germinated on the last day).

experiment. The studies were ended after 32 days. Seeds not germinating by day 32 were considered germinated on day 33.

Data were analyzed as means for each treatment combination (petri plate) using the Means, Correlation, and ANOVA procedures of SAS (SAS Institute).

Results. The first experiment was designed to determine optimum temperature for cold germination tests. Maximum germination percentage (84 and 83%) was observed at 22 and 18°C, respectively (Table 1). The minimum germination percentage (44%) was observed at 14°C. In the first experiment, 'Charleston Gray' had 71 % germination compared to 64% for 'Petite Sweet'.

The second experiment was to screen a diverse set of cultivars for germination speed at the chosen temperatures. In this experiment, 44 cultivars were tested at two different temerature, 14 and 18°C in the growth chamber. The maximum germination percentage (100%) was obtained for 17 cultivars: Blackstone, AU-Golden Producer, AU-Sweet Scarlet, Big Crimson, Black Diamond Yellow Belly, Calhoun Gray, Calsweet, Desert King, Fiesta, Klondike Striped II, Mickylee, Minilee, Peacock Shipper, Sugar Baby, Sultan F1, Super Gold, and Crimson Sweet (Table 2). Black Diamond Yellow Belly and Mickylee had high percentage germination (100%) at 18°C, but very low (0 to 5%) at 14°C.

At 14°C, 'Blackstone' and 'Starbrite' had the highest germination percentage (90 to 95%), while the other cultivars were below 80% (Table 2). Low germination percentage at low temperature has been reported in cucumber at 14°C (Harrington and Minges, 1954; Hegarty, 1977).

The watermelon cultivars used in this study were able to germinate at 14°C, but not at 10°C, during the 30 days used for the testing method. Some cultivars germinated rapidly, and with a high percentage at 14°C. If the trait is heritable, it could be transferred into elite cultivars to provide growers with more safety in early spring plantings. Improved cold germination ability may also provide better germination of triploid hybrids.

In the future, a larger collection of germplasm should be screened for germination at low temperature. Also, temperatures between 10 and 14°C should be evaluated to determine whether the best watermelon accessions can germinate below 14°C.

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Vine Length of A Diverse Set of Watermelon Cultivars

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Introduction. Breeding trials are carried out with the intent of maximizing the amount of information obtained while minimizing costs associated with trialing. Plots vary in the number of replications, years, and locations depending on whether yield and/or quality data is to be recorded (5). The size of a plot depends on the amount of seed available, equipment, labor, land, and the susceptibility of a crop to competition effects from neighboring plots. Smaller plot sizes allow greater numbers of lines to be evaluated with increased replication. However, they can be more susceptible to the effects of border competition if steps are not taken to group plots according to competing ability (1). Experiments and trials are suggested to have border rows around the plot and to harvest the center rows (net plots) unless border effects are not present (3, 6). Border rows can significantly increase the size and therefore costs of experiments or trials.

The need for border rows is crop specific and depends on competing ability differences of cultigens in adjacent plots. For watermelon, we were interested in vine length differences in relation to border competition effects. Studies on other cucurbits have noted main stem lengths to be positively correlated with yield per plant and have mostly additive genetic variance as did multiple lateral branching (2, 4). Long vined cultivars of cucumber have shown a tendency to reduce yields of adjacent cultivars (7). One study conducted on watermelon found only slight border competition effects due to differences in vine length. From their initial survey of breeders for extremes of vine length, the cultivars Sugar Baby and Petite Sweet were suggested as the shortest vined, Crimson Sweet as mid-vine length, and Charleston Gray, Jubilee, and Allsweet the longest vined. This same study found vine lengths for Charleston Gray to be 3.1 m (longest), 2.8 m for Crimson Sweet (intermediate), and 2.6 m for Sugar Baby (shortest) (8).

The objective of this study was to measure the vine length of a diverse set of watermelon cultivars that included bush type cultivars and varying flesh colors.

Methods. The experiment was conducted during the summer season of 2000 at the Horticultural Crops Research Station in Clinton, North Carolina.

The experiment had one replication, one measurement of vine length, one location and nineteen cultivars planted in single-row plots. Watermelon cultivars were either direct seeded into black plastic mulch and irrigated with plastic drip tape or into raised bare soil beds. Plots covered with plastic mulch were 3.7 m long, on 3.1 m centers with 0.6 m hills, and 2.4 m alleys at each end of the plot. Plots in bare soil were 6.1 m long, on 3.1 m centers with 1.5 m hills and 4.6 m alleys.

Nineteen cultivars were evaluated representing a range of vine lengths. Cultivars planted into plastic mulch included Garrisonian, Golden Honey, Klondike Striped 11, Mountain Hoosier, New Winter, and Stone Mountain. Cultivars on bare soil included Black Boy, Bush Charleston Gray, Bush Sugar Baby, Charlee, Fairfax, Klondike WR3, Picnic, Sugar Baby, Sugar Lee, Sweetheart, Tom Watson, Yellow Baby, and Yellow Crimson.

Cultivars were seeded on 1 May 2000 into plastic mulch and 25 May 2000 into bare soil. Three seeds of each cultivar were initially planted and were later thinned to one plant per hill. The experiment was conducted using recommended horticultural practices (Sanders, 1999). Plots were on raised beds of either bare soil or beds covered by black plastic mulch.

One measurement of vine length (in mm) was taken on 11 August 2000 for the cultivars on plastic and 17 August for bare soil, coinciding with fruit harvest stage. Vine length was measured from the base of each plant to the growing point of a main vine using a flexible metric tape. A main vine was chosen from those beginning close to the base of the plant and extending farthest from the base.

Results. Based on a replicated study in a field nearby, differences in vine length of less than 1 m probably are not significant between cultivars. When combined over both production methods (plastic

Table 1. Vine length of watermelon cultivars measured at Clinton, NC.

Mulch/Cultivar	Flesh color	Vine type	Length (m)
Black plastic			
Garrisonian	Red	Standard	4.8
Golden Honey	Salmon yellow	Standard	6.8
Klondike Striped 11	Red	Standard	7.4
Mountain Hoosier	Red	Standard	4.3
New Winter	Red	Standard	6.1
Stone Mountain	Red	Standard	5.7
Mean (black plastic)			*5.9*
Bare soil			
Black Boy	Red	Standard	2.8
Bush Charleston Gray	Red	Bush	2.7
Bush Sugar Baby	Red	Bush	1.2
Charlee	Red	Standard	5.6
Fairfax	Red	Standard	5.6
Klondike WR3	Red	Standard	4.4
Picnic	Red	Standard	5.6
Sugar Baby	Red	Standard	3.8
Sugar Lee	Red	Standard	2.9
Sweetheart	Red	Standard	2.8
Tom Watson	Red	Standard	4.4
Yellow Baby	Canary yellow	Standard	3.7
Yellow Crimson	Salmon yellow	Standard	3.5
Mean (bare soil, bush types)			*2.0*
Mean (bare soil, no bush types)			*3.8*

z Data are main vine length from 1 replication and 1 harvest date.

mulch and bare soil), the cultivar Klondike Striped 11 had the longest vines with a length of 7.4 m (Table 1). 'Bush Sugar Baby' was the shortest vined cultivar at 1.2 m. Although 'Bush Sugar Baby' had a shorter vine compared to cultivars having a standard vine length, the same could not be said for 'Bush Charleston Gray'. 'Bush Charleston Gray' (2.7 m) had a similar vine length compared to the standard vined cultivars Black Boy (2.8 m), Sugar Lee (2.8 m), and Sweetheart (2.8 m). Mean vine length was 5.9 m for cultivars on plastic mulch and 3.8 m for cultivars on bare soil.

Among cultivars planted into black plastic mulch, again 'Klondike Striped 11' had the longest vine length of 7.4 m while 'Mountain Hoosier' had the shortest (4.3 m). No bush type watermelon were planted in plastic mulch. Additionally no clear trend could be seen when comparing vine lengths based on cultivars differing in flesh color. The only salmon yellow cultivar planted to plastic mulch, 'Golden Honey', had a vine length of 6.8 m.

On bare soil, the cultivars Charlee, Fairfax, and Picnic all had the longest vine lengths with 5.6 m each. 'Bush Sugar Baby' had the shortest vine length of 1.2 m within all bare soil cultivars. Among non bush-type cultivars, 'Black Boy' and 'Sweetheart' were the shortest with 2.8 m each while 'Sugar Lee' had a similar vine length of 2.9 m. Cultivars with salmon yellow flesh had vine lengths close to the middle of the range in vine lengths for non-bush cultivars. Vine lengths for 'Yellow Baby' and 'Yellow Crimson' were 3.7 m and 3.5 m, respectively.

Conclusions. This survey of vine lengths showed a trend for higher vine lengths when cultivars were grown on plastic mulch than when grown on bare soil. Although some cultivars evaluated had a bush-type growth habit, they were not necessarily different in vine length than standard vine types. A large range in vine length was found for standard-vined cultivars (2.8 to 7.4 m) regardless of the growing environment. Even though slight border competition effects have been found for watermelon, caution should be undertaken when choosing cultivars to be included in each trial, especially the bush-type cultivars.

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Survey of Watermelon Trialing Methods Used by Breeders in the United States

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Introduction. We are in the process of developing efficient trialing methods for the breeding of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai). Efficient methods will provide maximum information for the resources used (6). Research in cucumber (*Cucumis sativus* L.) has provided information on optimum plot size (1, 3), multiple- vs. single-row plots (9), plot-end border effects (7), testing methods (5, 8, 10), and proper allocation of plots into replications, locations, seasons and years (4). However, little research has been done in watermelon on efficient trialing methods. It is also useful to have estimates of genetic variances, heritabilities, and genetic correlations for the important quantitative traits when designing optimum trialing methods (2).

Plot sizes used for watermelon trials range from single-plant hills with a low density of plants/ha to multiple-row plots of 0.1 ha in size. Small plots or hills can be used to evaluate many accessions, families, breeding lines, experimental hybrids, and cultivars (hereafter referred to collectively as cultigens). On the other hand, large plots provide data typical of production fields, with monoculture conditions, standard plant density, and a representative sampling of field variability. The optimum plot size is calculated using information about field variability, resources required, and number of cultigens that can be evaluated.

The objective of this study was to survey watermelon breeders in the U.S. to determine the methods being used for yield trials in the public and private sector.

Methods. Survey questions were sent to breeders with significant responsibilities for watermelon breeding in land grant universities and seed companies in the U.S.

One set of questions inquired about the layout of plots for use in preliminary and advanced evaluations of cultigens. Information was requested on the number of cultigens tested, row spacing, plot length, plants per plot, number of harvests, and number of replications used. A second set of questions focused on the methods used to identify the fruit associated with each plot at harvest. Questions were asked whether or not fruit type, wide row spacing, single- or multiple-row plots, vine tracing, vine training, and physical barriers were used.

Answers were summarized for public and private breeders. Means and ranges were used to summarize the important data, including plot length, row width, number of rows per plot, number of plants per row, number of harvests per plot, number of replications per trial, and number of cultigens evaluated.

Results. Many watermelon breeders use a three-stage system of trialing to test cultigens being developed in their programs: preliminary, intermediate, and advanced (data not shown). Cultigens being evaluated are generally direct-seeded or transplanted into the field plots.

The mean number of cultigens tested per trial for the breeders surveyed was 27 for public and 21.1 for private breeders (Table 1), with a range of 6 to 60. An average of 1.3 rows per plot and a mean plot length of 9.5 were found, along with 9.6 plants per row for public breeders. Breeders in the private sector used an average of 1.3 rows per plot, mean plot lengths of 15.8 and 19.1 plants per row. The mean number of plants per row also resulted from a wide range of responses (6 to 50). This range should not be surprising as the common spacing between plants within the row was 0.6 to 1.2 m, with a mean of 1 m for each breeding sector. Row widths had a mean of 2.9 m for public and 2.6 m for private breeders. Mean number of harvests ranged between 3.4 and 1.1 per season for public and private breeders respectively. Public breeders used an average of 4.0 replications and private breeders used 1.8.

Table 1. Description of trial layouts for watermelon breeding programs in the U.S.[z]

Breeding program	Total no. of programs	Statistic	Plot length (m)	Row width (m)	Rows /plot (no.)	Plants /row (no.)	Crop harvests (no.)	Trial reps (no.)	Cultigens /trial (no.)
University	4	Mean	9.5	2.9	1.3	9.6	3.4	4.0	27
		Low	6.1	2.7	1	6	2	3	6
		High	12.2	3.1	2	12.5	4	6	60
Seed Co.	9	Mean	15.8	2.6	1.3	19.1	1.1	1.8	21.1
		Low	8.2	1.5	1	6.5	1	1	6
		High	45.8	3.7	3	50	1.5	2	40
Combined	13	Mean	12.8	2.7	1	14	2	3	24

Table 2. Number of trials in watermelon breeding programs in the U.S. that use six different methods for separating the fruit in adjacent rows.[z]

Breeding program	No. of programs	Fruit type	Wide rows	Multiple rows	Trace vines	Train vines	Use barriers
University	4	3	3	2	2	1	0
Seed Co.	10	6	3	3	5	5	1
Combined	14	9	6	5	7	6	1

[z] Fruit type=cultigens with different fruit type are planted in adjacent rows; wide rows=wider rows are used for trials than are standard for growers; multiple rows=multiple rows per plot are used; trace vines=vines are traced from the fruit to its proper row; train vines=train vines for each row to keep from growing into adjacent row; use barrier=use barrier (for example, netting) to keep vines from each row in its assigned area.

Although plot spacings were not uniform over breeding programs, often breeders do not have total control over the management of their trials. Preliminary and intermediate trials are conducted on plots solely controlled by the breeder. However, advanced trials and some intermediate ones must be conducted off site using cooperating growers. Due to the high volume of plant material being evaluated for future release, breeders use the plant spacing of the cooperating growers.

From the data, smaller plots were commonly used with a limited number of cultigens being evaluated. This observation holds for each sector of plant breeders. Public and private breeders tended to use similar plot layouts. However, public breeders consistently used higher numbers of harvests and trial replications than their private counterparts. The use of smaller plots allows for more material to be tested on a smaller land area. One advantage of this method is the reduction of soil heterogeneity commonly found to affect the yields of large plots. Loss of information due to the use of small plots is compensated by the use of multiple locations and seasons (4). U.S. watermelon breeders commonly use close to ten locations and multiple seasons (data not shown).

One major problem of watermelon trials is to identify fruit belonging to adjacent rows. The majority of public breeders did this using a combination of wide row spacing and cultivars having different fruit type

assigned to adjacent plots (Table 2). About half of the private breeders used the combination of different fruit type, vine tracing and vine training (data not shown). Different fruit types in adjacent plots in the same row offered an additional advantage when vine overlap occurred in the alleys of multiple-row plots. The other methods were used at about the same frequency in the two sectors.

Use of a physical barrier was the least applied method of controlling vine growth or reducing fruit mix-up at harvest for public and private breeders. Survey responses for the use of vine tracing indicated its necessity especially when there was preference for allowing vines in a trial to grow undisturbed. Some breeders noted that vine growth and fruit set were altered when vines were trained or otherwise disturbed before harvest. Breeders also noted occasional problems with vine overlap within alleys at the ends of plots. However, responses indicated that vine overlap in general was a less significant problem in the western part of the U.S.

Conclusions. From this survey, we found the use of small plot sizes consisting of one to three rows per plot, few replications with multiple locations, low numbers of plants per plot, and one to two harvests to be commonly used in trials of U.S. private watermelon breeders. U.S. public watermelon breeders were found to use small plot sizes with one to two rows per plot, more replications, multiple locations, lower numbers of plants per plot then private breeders and multiple harvests. Although the use of small plots allows for more efficient use of space and labor resources, there is increased risk of border effect producing biased plot yields. To date, no research has been done regarding the need for border rows in plots of watermelon. Vine training methods found in this survey serve to reduce error while at the same time reduce border effect without adjusting row number. Adjusting the numbers or rows to create an artificial border could prove costly in terms of monetary costs, numbers of lines evaluated, and labor. The information found here will be used in future studies to make trialing procedures more efficient at maximizing the information gained while minimizing the use of resources.

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Variation Among Tropical Pumpkin (*Cucurbita moschata*) Cultivars in Susceptibility to Silverleaf

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Silverleaf is a distinctive disorder that has been observed on *Cucurbita pepo, C. moschata*, and *C. maxima* plants. Affected leaves are uniformly silver on the upper surface. Silverleaf is developmentally reversible so that silver and normal leaves may appear on the same plant.

The disorder was first noted in Israel in the 1960s and was first reported in the literature in the 1980s (Paris et al., 1987) and was associated with drought stress. Scattered reports of silverleaf from Miami-Dade County, Florida occurred prior to a major outbreak in 1987-88 season in several south Florida production areas (Maynard and Cantliffe, 1989). Symptoms are caused by feeding of silverleaf whitefly (*Bemisia argentifolii*) nymphs (Yokomi et al., 1990) and exacerbated by drought stress (Paris et al., 1993b).

Squash plant introductions from the U.S. National Germplasm System were evaluated for silverleaf resistance in naturally silverleaf whitefly infested fields in Puerto Rico in 1992, 1995, and 1996 (Wessel-Beaver, 1997). Those showing a high level of resistance included 14 of 420 *C. moschata* accessions, 69 of 350 *C. pepo* accessions and 7 of 405 *C. maxima* accessions. These results suggest the possibility of breeding for resistance to silverleaf.

Differential susceptibility to silverleaf among summer squash (*C. pepo*) cultivars has been reported (Paris et al., 1993a, 1993c). Cocozelle types had less silverleaf than crookneck, scallop, straightneck or zucchini types and vegetable marrow types were intermediate in silverleaf susceptibility. The least susceptible cultivars were 'Striato d' Italia', 'Kokacella', 'Bar' Oz' and 'Sih'i Lavan' that all originated in the Old World whereas cultivars developed in the new World were most susceptible to silverleaf.

Table 1. Incidence and severity of silverleaf in tropical pumpkin cultivars.

	Season		Season	
	1	2	1	2
Cultivar	(%)		Severity[1]	
Borinquen	90	100	4.0	4.0
Linea C Pinta	90	100	3.0	4.0
Soler	85	100	3.0	4.0
La Primera	35	75	1.6	1.0
La Segunda	25	0	1.8	0.0
L18-4	53	0	1.3	0.0

[1] 0 none to 4 severe silverleaf

Variation among tropical pumpkin cultivars in respect to silverleaf susceptibility was noted in fields that were naturally infested with the silverleaf whitefly in two consecutive seasons at Bradenton, Florida (Table 1). 'Borinquen', 'Linea C. Pinta', and 'Soler' which were developed in Puerto Rico had a high incidence of severe silverleaf in both seasons. 'Soler' was also found to be highly susceptible to silverleaf in studies conducted in Puerto Rico (Wessel-Beaver, 1997). On the other hand, 'La Primera', 'La Segunda', and L18-4 (a compact plant inbred) all developed in Florida had a much lower silverleaf incidence and plants were less severely affected.

Thus, the cultivar and geographical origin relationships noted (Paris et al., 1993a, 1993c) in *C. pepo* occurs in *C. moschata* as well. Although the reasons for the relationships are unclear at this time, they do offer guidance for selection of parents in breeding programs.

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Aggressiveness of Powdery Mildew Isolates on *Cucurbita maxima*

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Introduction. Pathogenicity of Czech isolates of cucurbit powdery mildew (*Erysiphe cichoracearum (Ec)* and *Sphaerotheca fuliginea (Sf))* on cucurbitaceous vegetables have been studied both on the level of their virulence and aggressivenes. Several *Ec* and *Sf* pathotypes previously described by Bertrand (2) were identified and new reaction patterns were recognized (4). Moreover some isolates were virulent also to watermelon (5) and one *Ec* isolate was virulent to *C. melo* MR-1 (6). The purpose of this study was to evaluate the aggressiveness of *Ec* and *Sf* isolates on *Cucurbita maxima* and to consider the influence of leaf side of this host genotype on the development of powdery mildew infection under controlled conditions.

Materials and Methods. A total of 15 *E. cichoracearum* and 8 *S. fuliginea* isolates collected from field cultures of cucurbits (*Cucurbita pepo*, *C. maxima*, *Cucumis sativus*) at seven distinct regions of the Czech Republic in 1997 were used for this study. Isolates were obtained from single spore lesions on leaves. Isolates were maintained *in-vitro* on the cotyledons of *Cucumis sativus* cv. Marketer according to methods described by Bertrand (2). For the determination of pathotypes the methods and differential plant genotypes proposed and kindly provided by Bertrand (2) were used. Czech *C. maxima* cv. Goliáš (accession number 09-H39-0137) originated from Gene Bank RICP in Olomouc (Czech Republic)

For the aggressiveness tests host material was prepared and inoculated separately from assays for pathopype determination. Leaf discs of 1.5 mm in diameter were cut out by a cork borer from well developed leaves of *C. maxima* cv. Goliáš plants 6-9 weeks old and placed either abaxial and adaxial sides on agar medium in Petri dishes. Aggressiveness of each isolate was evaluated on five discs in two replications for each side position. The inoculation was performed by spraying inoculum on discs. The inoculum was prepared by washing cucumber cotyledons with mycelium and conidia in water with addition of Tween. The number of conidia was approx. $55x10^3$ in 1 ml of suspension.

Intensity of sporulation on each disc was assessed visually 7, 10, 14 and 17 days after inoculation on a scale of 0 (no sporulation) to 4 (more than 75% of disc surface covered by mycelium) (7). The value of infection degree (ID) was counted for each isolate separately on the upper- (ID-u) and lower- (ID-l) leaf sides and a value of total infection degree (%TID) was counted from all subsequent evaluations as a percentage of maximum potential score. Data were treated statistically by one-way analyses of variance and Sheffe multiple range analyses in the programme Statgraphics (3).

Results and Discussion. Within isolates under study a total of three *Ec* pathotypes and two *Sf* pathotypes have been identified (Table 1). The average values of infection degree for *C. maxima* response to *Ec* were on the upper leaf side %TID-u = 34.3 (ab) and on the lower leaf side %TID-l = 22.3 (a), average values characterizing response of host genotype to *Sf* were on the upper leaf side %TID-u = 57.1 (b) and on the lower leaf side %TID-l = 37.2 (ab). Multiple range analyses (99%, Sheffe) proved differences between obtained values. Generally, the species *Ec* was less aggressive to *C. maxima* than *Sf* on both sides of leaf discs and the infection of both powdery mildew species (*Ec,Sf*) was more severe on the upper leaf disc side as compared to the response of the lower disc side. The curves characterizing infection development on each leaf side were similar for both powdery mildew species (Figure 1). During first seven days after inoculation the infection development of both *Ec* and *Sf* was faster on the upper leaf side, than during next three days this process accelerated on the lower side.

Table 1. Aggressiveness of powdery mildew isolates on *Cucurbita maxima* cv. Goliáš

Isolate number	Pathotype[x]	TID(%)[y] upper	TID(%)[y] lower	Host plant[z]	Region
E. cichoracearum					
3/97	nd	7.5	3.8	*C. maxima*	Olomouc
40/97	AB1B2CCm	15.0	3.8	*C. pepo* SC	Prostějov
15/97	nd	20.0	0.0	*C. pepo* ZU	Prostějov
30/97	nd	25.0	1.3	*C. pepo* ZU	Prostějov
41/97	AB1B2CCm	25.0	25.0	*C. pepo* ZU	Prostějov
19/97	AB1B2CCm	27.5	13.8	*C. maxima*	Praha
17/97	ACm	36.3	26.3	*C. pepo* PU	Prostějov
44/97	AB1B2CCm	36.3	42.5	*C. pepo* VM	Olomouc
29/97	AB1B2CCm	45.0	6.3	*C. pepo* ZU	Olomouc
23/97	AB1B2CCm	46.3	54.7	*C. maxima*	Prostějov
20/97	AB1B2CCm	67.5	33.7	*C. sativus*	Olomouc
38/97	AB1B2CCm	68.8	28.8	*C. pepo* ZU	Brno
25/97	ACCm	70.0	35.0	*C. pepo* ZU	Brno
average		**34.3 ab[1]**	**22.3 a[1]**		
S.fuliginea					
13/97	AB1CCm	40.0	17.5	*C. pepo* ZU	Kolín
36/97	AB1-CCm	45.0	42.5	*C. maxima*	Šumperk
32/97	nd	48.4	26.3	*C. pepo* ZU	Olomouc
34/97	AB1CCm	55.6	71.3	*C. pepo*	Olomouc
16/97	nd	58.8	12.5	*C. pepo* ZU	Olomouc
26/97	AB1CCm	60.0	77.5	*C. maxima*	Olomouc
37/97	AB1CCm	70.0	10.0	*C. pepo* ZU	Kroměříž
10/97	AB1Cm	78.8	40.0	*C. maxima*	Olomouc
average		**57.1b[1]**	**37.2ab[1]**		

[x] compatible reaction on:

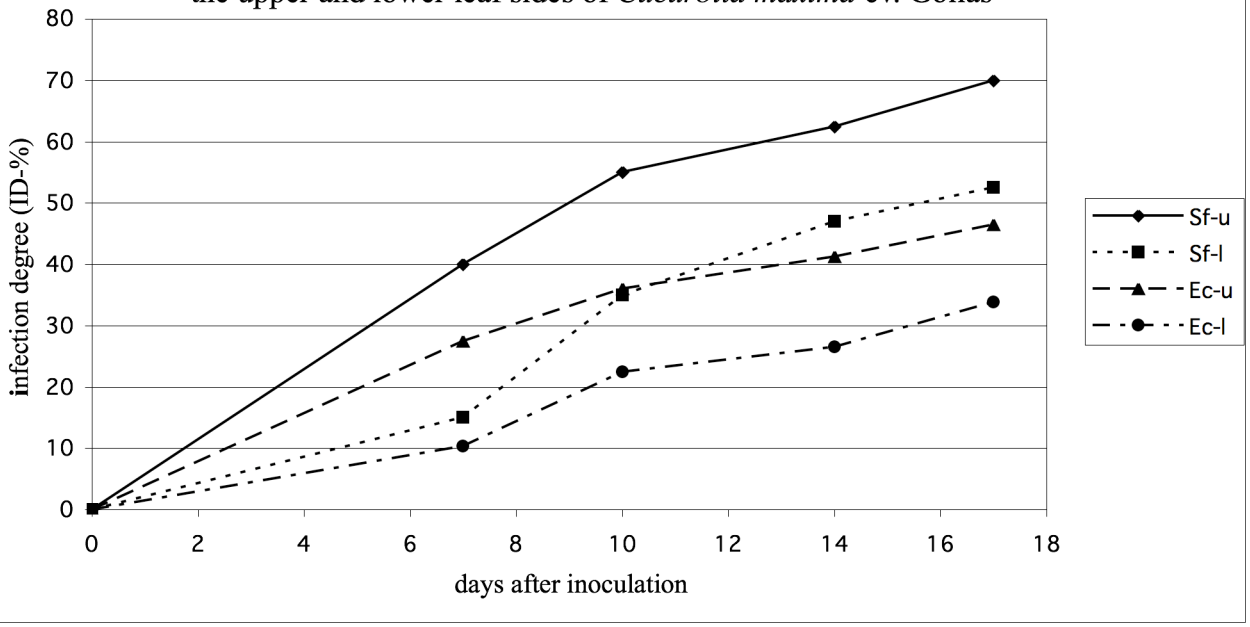
A	*C. sativus* cv. Marketer	C	*C. pepo* cv. Diamant F1
B1	*C. melo* Védrantais	Cm	*C. maxima* cv. Goliáš
B2	*C. melo* PMR 45	D	*C. lanatus* cv. Sugar Baby

[y] Total infection degree on lower and upper leaf side of *C. maxima*

[z] *C. pepo* morphotype according to Paris (9): PU pumpkin, SC scallop, VM vegetable marrow, ZU zucchini

[1] Homogeneous groups (Multiple range analyses, 99%, Sheffe)

Figure 1. Infection development of *E. cichoracearum* and *S. fuliginea* on the upper and lower leaf sides of *Cucurbita maxima* cv. Goliáš



A large variability of aggressiveness within individual isolates of both fungi was found. Similar phenomenon was mentionned also by Bardin et al. (1). *Ec* isolates with different virulence (e.g. 25/97 and 38/97) expressed very similar aggressiveness on both leaf sides of *C. maxima* and, on contrary the aggressiveness of *Ec* isolates virulent to the same spectrum of differential plant species (e,g, 38/97 and 40/97) was quite different. *Sf* isolates 34/97 and 37/97 with the same reaction pattern on differential genotypes proved different aggressiveness on both sides of *C. maxima* leaf discs. Three isolates of *Ec* and two isolates of *Sf* were more aggressive on the lower leaf side than on the upper one (Table 1).

Differences in aggressiveness within individual isolates were not associated with original host plant species, region of their collecting and/or their virulence (pathotype). Only one *Ec* isolate was not virulent on the lower leaf side. Such reaction would be further studied and considered to be used as a marker for partial resistance of *Cucurbita maxima* cv. Goliáš as proposed by Leibovich et al. (8) for some other *Cucurbita* species.

Explanation of the role of specific morphological, biochemical and physiological features of each leaf side and co-evolution with powdery mildews could provide general conclusions about specific host - pathogen interactions. Aggressiveness of powdery mildew isolates is further studied on a broader spectrum of host genotypes.

Till now, *Ec* is a predominating powdery mildew species on cucurbits in the Czech Republic (4). A very high aggressiveness of *Sf* isolates on *C. maxima* can influence a broader geographic distribution of this powdery mildew species on this territory.

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Evaluation of the Cross Eskandarany x Whitaker for Powdery Mildew Resistance (PMR), Zucchini Yellow Mosaic Virus Resistance (ZYMR) and Some Yield Characters

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Introduction: Cucurbits play a significant role in human nutrition, especially in tropical countries where their consumption is high. Cucurbit crops constitute a major portion of vegetables and are grown in different regions of the Sudan. Squash is a promising export crop which can be readily produced at a low cost during the winter season of Sudan.

Zucchini yellow mosaic virus (ZYMV) and powdery mildew (PM) are major diseases in Sudan, causing significant yield losses (1,2). Summer production of squash resulted in low yield caused by failure of fruit development (5). The popular commercial cultivar of squash in Sudan is 'Eskandarany' which is susceptible to ZYMV and PM . The cultivar Whitaker is a summer squash released in 1998 at Cornell University (USA). Whitaker is resistant to four important diseases: ZYMV, papaya ring spot virus (PRSV), cucumber mosaic virus and PM (10).

Two aggressive isolates of ZYMV, Su19 and Su4, were reported in Sudan (4). The isolate Su19 is an aggressive isolate widely distributed in central Sudan where the field test was done. It incites typical yellow mosaic, deep foliar serration, blisters, deformation and plant stunting on the susceptible cv. Eskandarany. Powdery mildew caused by *Sphaerotheca fuliginea* (Schlecht. Ex Fr. Pol), race 0, 1 and 2 were reported (3), with seasonality of race prevalence. Resistance to PM was reported in wild *Cucurbita lundelliana* (12). This resistance was found to be conferred by a single dominant gene. Another wild squash, *C. martinezii,* was reported to be resistant to PM and this resistance was transferred to *C. pepo* (7). A single incompletely dominant gene *zym* plus some modifiers has been reported to confer resistance to ZYMV (8).

The objectives of this study were to determine (1) the inheritance of resistance to PM and ZYMV, and (2) the yield potential of the parents in cross combination.

Materials and Methods: Cultivar Eskandarany was selfed for four generations to ensure homozygosity. Six generations P_1 (Eskandarany), P_2(Whitaker), F_1, F_2, BCP_1, BCP_2 were generated at University of Gezira farm. The six generations were evaluated during the winter season of 2000/2001. The experimental design used was a randomized complete block design with three replicates . Evaluation of powdery mildew was done under natural conditions of infection. A scale of 1 to 9 was used (2), where 1 = stem: fungal growth with luxuriant sporulation; leaf: completely covered with fungal growth with luxuriant sporulation; 9 = stem and leaf free of fungal growth. Evaluation was done once at the end of the season and when the susceptible parent (P_1) was completely infected. Melon genotypes obtained from INRA, France were used to identify the PM causal agent (Table 1).

Evaluation for ZYMV was done under field conditions in season 1998/99 in Sudan. A scale of 1 to 9 was used, where 1=the plant is severely infected and is showing shoestringing symptoms, 9= no apparent symptoms of the virus. Mechanical inoculation with ZYMV-Su19 was done in the green house and ELISA testing at INRA, Montfavet, France. Three yield characters were measured:

1. number of days to flowering
2. ovary length at flowering (cm).
3. number of days from flowering to harvest (marketable size).

Table 1: Observed reaction of melon genotypes to powdery mildew.

Iran H	Nantais oblong	PMR 45	WMR 29	MR1	PMR5	PI 124112	PI 414723	Edisto
S	S	R	R	R	R	R	R	R

Table 2. Inheritance of powdery mildew resistance in cv. Whitaker (*Cucurbita pepo* L.).

Generation	No of plants	[z]R	S	Expected Ratio	X^2	P
P_1	35	0	35	0:1		
P_2	16	16	0	1:0		
F_1	38	38	0	1:0		
F_2	335	272	62	3:1	1.674>	99%
BCP_1	36	17	19	1:1	0.014>	99%
BCP_2	35	35	0	1:0		

[z]R: resistant, S: susceptible

Table 3. Generation means of ovary length, days to flowering and days to harvest marketable fruits.

Generation	Ovary length(cm)	Days to flowering	Days to harvest
[z]P_1	5.6±0.13	55.5±0.79	6.2±0.13
P_2	7.6±0.23	56.2±1.16	3.4±0.2
F_1	8.3±0.31	45.8±0.9	5.1±0.2
F_2	7.8±0.08	52.1±0.46	4.7±0.07
BCP_1	6.8±0.2	53±1.09	4.8±0.2
BCP_2	8.2±0.21	49.9±1.21	4.8±0.24

P_1: Eskandarany, P_2: Whitaker

For generation mean analysis, six parameter model (6) was used (m=mean, d=additive effect, h=dominance effect, i=additive x additive, j=additive x dominance, l= dominance x dominance). Narrow sense heritability (h^2_N) was estimated according to the partition of variance (11).

Results and Discussion: The Chi-square analysis revealed a good fit for a single completely dominant gene hypothesis for resistance to powdery mildew (Table 2) . According to the observed reaction of the differential genotypes race 1 of *Sphaerotheca fuliginea* is prevailing (Table 1). Nantais oblong is known to be resistant to *Erysiphe Cichoracearum* and race 0 of *S. fuliginea* whereas PMR45 is resistant to race 1 and susceptible to race 2 of *S. fuliginea*.

The cv. Whitaker displayed a high level of resistance to zucchini yellow mosaic virus (ZYMR=8) under natural infection conditions. Plants showed very light mosaic producing fruits with normal shape, size and color. The F_1 (Eskandarany x Whitaker) showed an intermediate level of resistance (ZYMR=5). The plants displayed a high level of mottling on leaves but they were vigorous and produced marketable fruits. Upon inoculation with the isolate Su 19 the cv. Whitaker displayed clear mosaic. The ELISA test confirmed the presence of the virus, but the virus

concentration is lower than that of the cv. Eskandarany. Hence, cv. Whitaker possesses a high tolerance to ZYMV-Su19. No source of immunity for summer squash to natural infection with ZYMV is reported (10).

The cv. Whitaker is not well adapted to Sudan conditions. But the F_1 Eskandarany x Whitaker showed a high level of tolerance to high temperature and it was vigorous and productive . Thus, the F_1 can be released as a summer F_1 hybrid. Since Whitaker derived its resistance to ZYMV from *C. ecuadorensis*, pyramiding genes with that of our breeding lines that derived their resistance to ZYMV from Nigerian Local is under way in an attempt to breed cultivars with a better level of resistance to ZYMV-Su19

Means of the three yield characters are shown in table 3. It is evident that the F_1 is showing heterotic effects in having a longer ovary and being earlier by about 10 days. The generation mean analysis based on the six parameter model indicates that additive and dominance gene effects were significant ($P>0.05$) for number of days to flowering with h^2_N =0.66. For ovary length additive and additive x additive effects were significant with h^2_N =0.65 while dominance and additive x dominance were significant for number of days to marketable size with h^2_N =0.2.

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Same Gene for *Bush* Growth Habit in *Cucurbita pepo* ssp. *pepo* as in *C. pepo* ssp. *ovifera*

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Cucurbita pepo L. is highly polymorphic for reproductive as well as vegetative characteristics. Growth habit is among the variable vegetative traits. Many forms have a spreading, viney growth habit, with long, thin internodes. Others have a compact, bushy growth habit with short, thick internodes. Only one locus, designated *Bu*, has heretofore been identified as conferring bush or vine growth habit, with the allele for bushiness, *Bu*, incompletely dominant to the allele for vininess, *bu*. However, as the degree of vininess and bushiness can vary, other loci must exist that modify the expression of *Bu*.

On the basis of fruit shape, edible-fruited *C. pepo* have been considered as eight cultivar-groups: Acorn, Cocozelle, Crookneck, Pumpkin, Scallop, Straightneck, Vegetable Marrow, and Zucchini (4). On the basis of allozyme variation and seed morphology, each of these groups is considered to belong to one of two subspecies: *C. pepo* ssp. *pepo* or *C. pepo* ssp. *ovifera* (1). Cultigens of each of the two subspecies apparently were developed independently and in different regions thousands of years ago (1). Nonetheless, some horticulturally valuable characteristics not present in wild forms but present in cultigens have existed in both subspecies for quite some time. One of these is bush growth habit, a characteristic that facilitates multiple harvesting. Bush growth habit was illustrated in botanical herbals of the 16th century in forms of both subspecies (5). It seems possible that bush mutants were selected by indigenous peoples separately in *C. pepo* ssp. *pepo* and *C. pepo* ssp. *ovifera*. Therefore, it appears worthwhile to determine if these bush forms are derived from mutations at separate loci or if the same mutation occurred in the two subspecies.

As bushiness and vininess can vary in degree, it seemed to us imperative to develop two near-isogenic lines carrying bush growth habit, one carrying the bush gene from *C. pepo* ssp. *pepo* and the other carrying the bush gene from *C. pepo* ssp. *ovifera*. This was accomplished by using 'Fordhook Zucchini' (*C. pepo* ssp. *pepo* Zucchini Group) as one donor parent of bush growth habit (3) and 'Bush Ebony' (*C. pepo* ssp. *ovifera* Acorn Group) as the other. The recurrent parent was the viney 'Vegetable Spaghetti' (*C. pepo* ssp. *pepo* Vegetable Marrow Group). The F_1s of crossing 'Fordhook Zucchini' and 'Bush Ebony' with 'Vegetable Spaghetti' were semi-bush, thus bush growth habit was incompletely dominant, as previously reported (2). The bush characteristic was introgressed from the donor parents to 'Vegetable Spaghetti' by six generations of backcrossing, selecting for semi-bush growth habit in each generation. This was followed by two to three generations of self-pollination, until true-breeding bush lines were obtained.

The two true-breeding bush lines, 85a-30-45-17 (bush habit from 'Fordhook Zucchini') and 823a-13-20-17 (bush habit from 'Bush Ebony'), did not appear to differ from one another in expression of bush growth habit and were crossed. The F_1 plants were of bush habit that was of no greater or lesser expression than that of the parents. Some F_1 plants were then testcrossed to an inbred, designated VSP-4-10-4, of the viney 'Vegetable Spaghetti', or to a viney near-isogenic line of 'Vegetable Spaghetti' designated 85k-9-107-2 (6). All of the 39 progeny of these testcrosses that were observed had semi-bush growth habit (Table 1). Apparently, the gene for bush growth habit in *C. pepo* ssp. *pepo* 'Fordhook Zucchini' is identical to that in *C. pepo* ssp. *ovifera* 'Bush Ebony'. Had there been two genes for bush habit at separate loci, then one or more viney individuals should have occurred among the 39 plants observed.

Table 1. Parental lines and F_1s observed for growth habit. Sown 8 March 2000 in flats, transplanted to the field 28 March, observed 17 May.

Line	No. plants	Growth habit
P_1, VSP-4-10-4	7	Vine
P_2, 85a-30-45-17	13	Bush, derived from FZU
P_3, 823a-13-20-17	8	Bush, derived from BEB
P_4, 85k-9-107-2	8	Vine
1014 ($P_2 \times P_3$)	8	Bush
1033 ($P_2 \times P_3$) $\times P_1$	16	Semi-bush
1033Ra $P_1 \times (P_2 \times P_3)$	12	Semi-bush
1034 ($P_2 \times P_3$) $\times P_4$	7	Semi-bush
1034Ra $P_4 \times (P_2 \times P_3)$	4	Semi-bush

FZU = Fordhook Zucchini, BEB = Bush Ebony

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Relationship between Fruit Shape and Seed Yield in *Cucurbita pepo*

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Cucurbita pepo is a very diverse species for fruit shape and size. The domestication of the species started thousands of years ago in North America from native small, round, usually bitter-flesh gourds. The initial use of these gourds by humans appears to have been consumption of the seeds, and thus the first steps of human selection were directed toward increasing fruit and seed size (1, 7). The fruit flesh could be consumed only after several cycles of boiling it to remove the bitter cucurbaticins. Subsequently, variants having non-bitter fruit were selected, resulting in the development of the pumpkins. The first pumpkins may have had a dual usage, for consumption of their seeds and consumption of their immature fruits, just as the land-race pumpkins inly Mexico and Guatemala do today. Later, selection for thicker, more starchy, and less fibrous fruit flesh allowed for consumption of the mature fruits, which today is the common culinary usage of pumpkins in the United States and Canada. The seeds, nonetheless, have some importance in economically developed countries as a high nutritive snack food and in the production of pumpkin seed oil (1, 6).

The great economic value of *C. pepo* today is based mainly on the culinary use of the young, immature fruits, often referred to collectively as summer squash. Summer squash deviate in shape from the roundness of their gourd and pumpkin ancestors (4). Selection has been geared toward deviation from the 1:1 length-to-width ratio in order to achieve a narrower, smaller volume of the soft placenta (endocarp). This resulted in the development of the flat (length-to-width ratio less than 1:1) scallop squash in North America prior to the European contact with that continent 500 years ago. After introduction of *C. pepo* to Europe, the culinary use of the young fruits began to be appreciated there. Selection for improved culinary traits occurred, most notably toward deviation from the 1:1 ratio in the direction of longer fruits, resulting in the development of the vegetable marrows (short, tapered cylindrical fruits), followed soon afterward by the cocozelles (long or very long, bulbous cylindrical fruits), and much more recently, by the zucchinis (long, uniformly cylindrical fruits) (5).

In a previous study (2) we tried to find relationships among fruit dimensions, seed cavity dimensions and seed yield in *C. pepo* ssp. *pepo*, which contains the Pumpkin, Vegetable Marrow, Cocozelle, and Zucchini cultivar-groups (5). In that work, we had included accessions that greatly differed in fruit weight and did not observe a consistent trend of seed yielding among these groups of cultivars. We decided to investigate further, this time using accessions that were more similar in fruit size. We chose 16 accessions, four from each of these four cultivar-groups (Table 1). We grew four replicates of four plants per accession at the Newe Ya'ar Research Center (northern Israel) in the summer season of 2000. Each plant was hand-pollinated to form 1-3 fruits/plant. The fruits were harvested 45 days past anthesis. Each fruit was weighed and then divided into three equal parts by length. The three parts will herein be referred to as the center, stylar end, and peduncular end. Each part was weighed and the seeds it contained were weighed and counted.

Mean fruit length (stylar end to peduncular end) increased and mean fruit diameter (equatorial) decreased in the order: pumpkin, vegetable marrow, zucchini and cocozelle (Table 2). The length-to-diameter ratio was a reliable parameter for differentiating among the four cultivar-groups.

The Pumpkin Group had more seeds per fruit than any other (Table 3). The Zucchini Group had the smallest seeds. The range of differences among cultivar-groups in seed number per fruit and in mean seed weight was 63 and 23%, respectively. The differences in the two seed-yield components resulted a clear differentiation of the four cultivar-groups in respect to seed yield. The Pumpkin Group had the highest yield, the Zucchini Group the lowest, and the Cocozelle Group and the Vegetable Marrow Group were intermediate.

Table 1. List of cultivar-groups, accessions, abbreviations, and origins.

Cultivar-group	Accession name	Abbreviation	Origin
Pumpkin	Cinderella	CIN	U.S.A.
Pumpkin	Early Sweet Sugar Pie	ESS	U.S.A.
Pumpkin	Porqueira	PRQ	Portugal
Pumpkin	Tondo Chiaro di Toscana	TOC	Italy
Vegetable Marrow	Beirut	BEI	Israel
Vegetable Marrow	Blanche non-coureuse	BNC	France
Vegetable Marrow	Long Green	LOG	U.K.
Vegetable Marrow	Verte Petite d'Alger	VPA	France
Cocozelle	Lungo Bianco di Sicilia	LBS	Italy
Cocozelle	Long Cocozelle	LCO	U.S.A.
Cocozelle	Lunga di Toscana	LUT	Italy
Cocozelle	Striato d'Italia	STI	Italy
Zucchini	Black Beauty	BBU	U.S.A.
Zucchini	Black Zucchini	BZU	U.S.A.
Zucchini	Nero di Milano	NER	Italy
Zucchini	Nano Verde di Milano	NVM	Italy

Table 2. Fruit dimensions in 16 accessions of *Cucurbita pepo*.

Cultivar-group	Cultivar	Fruit length (cm)	Fruit diameter (cm)	Length /Diameter
Pumpkin	CIN	14	19	0.71
Pumpkin	ESS	11	15	0.74
Pumpkin	PRQ	22	20	1.14
Pumpkin	TOC	11	17	0.69
Pumpkin	Mean	14	18	0.82
Vegetable Marrow	BEI	28	11	2.47
Vegetable Marrow	BNC	23	14	1.63
Vegetable Marrow	LOG	34	13	2.54
Vegetable Marrow	VPA	31	13	2.41
Vegetable Marrow	Mean	29	13	2.26
Cocozelle	LBS	41	8	5.02
Cocozelle	LCO	42	9	4.48
Cocozelle	LUT	49	11	4.60
Cocozelle	STI	61	10	6.37
Cocozelle	Mean	48	9	5.12
Zucchini	BBU	30	9	3.59
Zucchini	BZU	41	10	4.01
Zucchini	NER	40	10	3.88
Zucchini	NVM	39	11	3.48
Zucchini	Mean	37	10	3.74

Table 3. Seed yield per fruit and seed yield components in four cultivar-groups of *Cucurbita pepo*.

Cultivar-group	Cultivar	Seeds per fruit	Mean seed weight (mg)	Seed yield per fruit (g)
Pumpkin	CIN	372	68	25.30
Pumpkin	ESS	513	99	50.79
Pumpkin	PRQ	373	185	69.00
Pumpkin	TOC	247	128	31.62
Pumpkin	Mean	376	120	45.12
Vegetable Marrow	BEI	102	158	16.12
Vegetable Marrow	BNC	399	124	49.48
Vegetable Marrow	LOG	239	156	37.28
Vegetable Marrow	VPA	336	88	29.57
Vegetable Marrow	Mean	269	131	35.24
Cocozelle	LBS	303	94	28.48
Cocozelle	LCO	392	102	39.98
Cocozelle	LUT	211	171	36.08
Cocozelle	STI	267	163	43.52
Cocozelle	Mean	293	132	38.68
Zucchini	BBU	150	80	12.00
Zucchini	BZU	149	134	19.97
Zucchini	NER	191	129	24.64
Zucchini	NVM	429	85	36.46
Zucchini	Mean	230	107	24.61

Table 4. Seed yield distribution among the center, stylar end, and peduncular end in the fruits of four cultivar groups of *Cucurbita pepo*.

Cultivar-group	Cultivar	Seed yield (%) Stylar	Center	Peduncular
Pumpkin	CIN	25	42	32
Pumpkin	ESS	26	43	30
Pumpkin	PRQ	36	42	21
Pumpkin	TOC	31	58	10
Pumpkin	Mean	29	46	23
Vegetable Marrow	BEI	55	43	1
Vegetable Marrow	BNC	35	49	15
Vegetable Marrow	LOG	49	42	8
Vegetable Marrow	VPA	44	49	7
Vegetable Marrow	Mean	46	46	8
Cocozelle	LBS	70	29	0
Cocozelle	LCO	65	35	0
Cocozelle	LUT	88	12	0
Cocozelle	STI	89	10	0
Cocozelle	Mean	78	21	0
Zucchini	BBU	41	56	3
Zucchini	BZU	36	64	0
Zucchini	NER	41	56	3
Zucchini	NVM	49	47	3
Zucchini	Mean	42	56	2

The four cultivar-groups differed markedly in seed-yield distribution in the fruit (Table 4). In the Pumpkin Group, about half of the seed yield was produced in the central portion of the fruit, with about one-quarter of the yield each in the stylar and peduncular portions. In the Vegetable Marrow Group and the Zucchini Group, nearly all of the seed yield was produced in the central and stylar portions of the fruit. In the Cocozelle Group, over three-quarters of the seed yield was produced in the stylar portion, with none at all in the peduncular portion.

The results (Table 3) indicate that seed yield per fruit in *Cucurbita pepo* ssp. *pepo* has decreased over the course of history with highest yield in the most ancient type, the Pumpkin Group, and the lowest yield in the most modern type, the Zucchini Group. The decrease in seed yield per fruit is generally true also over the transition from round (pumpkin) to wedge-shaped (vegetable marrow) to long (cocozelle and zucchini) fruits, except that the Cocozelle Group, even though it has the longest fruits, produced a similar or higher yield than the Vegetable Marrow Group. This can be attributed to the bulbous stylar end of the cocozelles, in which nearly 80% of their seeds are produced. The dominant seed yield component which determined the differences in seed yield among cultivar-groups was the seed number per fruit. The pumpkins had the highest seed number and the most even distribution of seeds in the fruit. In the other cultivar-groups, there was a trend toward decreased seed number, especially in the peduncular end of the fruit. This phenomenon became increasingly apparent as the length-to-width ratio increased, quite consistent with observations made in cucumbers (3).

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The Use and Development of Molecular Breeding Tools in *Cucurbita*: A Literature Review

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Tools for molecular mapping and marker-assisted selection are being increasingly applied to the genus *Cucurbita*. However, much of the work which has already been done has remained unpublished, or has been published in journals of limited circulation, such that many who work with *Cucurbita* may not be aware of it. This review of the literature to date is intended to provide some background for current work.

The development of molecular tools for use in breeding *Cucurbita* species is still in the early stages, particularly when compared to cereal crops such as corn and wheat, and other vegetable crops such as tomatoes and lettuce. Even the *Cucumis* species *C. melo* and *C. sativus* are far ahead of *Cucurbita*. One reason for the late development of molecular tools in *Cucurbita* is that all species have twenty pairs of relatively short chromosomes. Using flow cytometry, Arumuganathan and Earle (1) determined that the haploid genome of zucchini (*C. pepo*) is approximately 500 million base pairs long. A typical nucleus (2n) contains 1.04 – 1.08 picograms of DNA. Most morphological traits appear to be unlinked, and many markers are required to adequately map the genome. *Cucurbita* species are of limited economic significance in developed countries, and most *Cucurbita* researchers work on several crops. The large size of the plants makes them ill-suited to genetics studies, and some types require a very long growing season.

No linkage map of any *Cucurbita* species existed prior to the development of molecular mapping. In the 1980s the use of isozymes revealed that *Cucurbita* is an ancient allotetraploid (28). Isozymes also provided a marker linked to one of the complementary genes responsible for the expression of WMV resistance in crosses between *C. maxima* and *C. ecuadorensis* (27).

Many of the species in *Cucurbita* can be successfully crossed, particularly if embryo rescue is used. However, the F1 and later generations of the interspecific crosses are frequently sterile or exhibit reduced fertility (22). Isozymes have been used to determine if the reduced fertility was a result of chromosomal rearrangement in crosses between *C. maxima* and *C. ecuadorensis*. Wall and Whitaker (25) examined the inheritance of leucine aminopeptidase and esterase isozymes in a set of *C. ecuadorensis* x *C. maxima* crosses and concluded that the chromosomal structure of the two species differed in the region of the esterase locus. Weeden and Robinson (26) examined the inheritance of twenty isozymes in the cross *C. maxima* x *C. ecuadorensis*. They used their data to build the first map of *Cucurbita*. It was based on the F2 of the cross *C. maxima* x *C. ecuadorensis*, and contained 11 isozyme loci in five linkage groups. From the isozyme map Weeden and Robinson (26) were able to determine that the significant decrease in fertility of the F2 and backcross generations of crosses between *C. maxima* and *C. ecuadorensis* was not a result of minor chromosomal rearrangements.

Restriction Fragment Length Polymorphisms (RFLPs) have not been much used in *Cucurbita* because of the time and expense involved in creating the probes. However, ribosomal (8, 24) and chloroplast DNA has been studied, and the chloroplast genome has been mapped (20). The chloroplast map was constructed using gene-specific probes from other species; this is possible because the chloroplast genome is highly conserved. Wilson et al. (29) used restriction enzymes and cloned chloroplast fragments to study the chloroplast DNA diversity of 15 species of *Cucurbita*. Their conclusions agreed with those obtained through interspecific crossing studies and standard taxonomy. They also determined that the annual *Cucurbita* species evolved from the perennial species. Havey et al. (10) used RFLPs to study the transmission of the chloroplast and mitochondrial genomes in cucurbits. They concluded that both organelle genomes were maternally transmitted in *Cucurbita*, unlike in *Cucumis* where the mitochondrial genome is paternally transmitted.

Lee et al. (17) developed a RAPD map of an F2 population of a *C. pepo* x *C. moschata* interspecific hybrid. They screened the parents with 70 10-mer primers from the University of British Columbia (UBC 501-570); 15 of the primers were polymorphic between the parents. These fifteen primers were used to amplify DNA from 40 F2 individuals, resulting in 58 RAPD bands. Forty-seven reproducible markers were used to build the map; 28 markers were mapped into five linkage groups. No morphological traits or other types of markers were included on the map. The map of Lee et al. (17) was small, based on a small number of progeny, and was published in the Korean Journal of Horticulture, with the result that it is not well known in the *Cucurbita* community. Furthermore, they selected arbitrary identifiers for the markers on their map, rather than following the standard practice of identifying the markers by the primer and the band size in base pairs. This further prevents comparisons between their map and other *Cucurbita* maps. The map of Lee et al (17) is currently the only published molecular map of *Cucurbita*. Data were collected for a random amplified polymorphic DNA (RAPD) map on a *C. maxima* x *C. ecuadorensis* population, but the map was never published (N. Weeden, personal communication).

RAPDs and other molecular marker technologies have been used to do DNA fingerprinting analysis within and between *Cucurbita* species. Jeon et al. (11) used RAPDs to distinguish among Korean cultivars of *C. pepo* and *C. moschata*, while Youn et al. (30) used RAPDs to study the genetic relationships among South Korean landraces of *C. moschata*. Stachel et al. (23) used RAPDs to estimate genetic diversity among commercial inbred lines of Austrian oilseed pumpkin, *C. pepo* var. *styriaca*. Gwanama et al (9) used RAPDs to determine the genetic variability present in the *C. moschata* landraces of south-central Africa. Baranek et al. (2) used RAPDs to study the genetic diversity within and between species of *C. pepo*, *C. moschata*, and *C. maxima*. All the researchers found RAPDs to be effective for determining the relatedness of different *Cucurbita* accessions. Katzir et al. (13) used microsatellite-anchored sequences as primers (ISSR) to classify cultivars of *C. pepo*. No microsatellites specifically designed for *Cucurbita* have been published, but polymorphisms among *Cucurbita pepo* accessions have been detected using SSRs developed for *Cucumis* (14, 15).

Polymorphism levels in *Cucurbita* are moderate. Stachel et al. (23) found 116 polymorphic markers by screening a set of 20 inbred lines of oilseed pumpkin (*C. pepo* var. *styriaca*) with 34 RAPD primers. Baranek et al.(2) found 42.5% marker polymorphism among six Austrian *C. pepo* genotypes. Katzir et al. found that 14% of the *Cucumis* SSR primers (15) and 82% of ISSR markers (13) were polymorphic among cultivar groups of *C. pepo*. Youn et al. (30) found that 18.6% of markers were polymorphic among *C. moschata* landraces. Brown and Myers (4) found 14% marker polymorphism between temperate and tropical *C. moschata* lines. Baranek et al. (2) found 64.1% marker polymorphism among a geographically disparate collection of *C. moschata* accessions. They found 55.9% marker polymorphism among a disparate collection of *C. maxima*.

Marker assisted selection is little-used in *Cucurbita* breeding. Brown and Myers have identified RAPD markers linked to several morphological traits in an interspecific cross between a *C. pepo* summer squash and *C. moschata* 'Nigerian Local' (see article in this issue) but they are not yet in a form that will be directly useful to breeders. Two groups have been working on identifying molecular markers linked to ZYMV resistance from 'Nigerian Local' introgressed into *C. pepo* (2, 19) but no markers have been published.

A number of *Cucurbita* genes have been characterized at the molecular level and cloned. However, this work has been done entirely by molecular geneticists interested in the control and functioning of pathways common to many plants. Thus the genes cloned have been of little direct use to squash breeders. Researchers in the laboratory of G. A. Thompson at the University of Arizona have studied and cloned the genes encoding important proteins in the phloem transport system of *C. maxima* (3, 6, 18). Other recently cloned genes in *Cucurbita* include a calcium-dependent protein kinase from zucchini (7), a class-3 chitinase (16), a glyoxysomal malate dehydrogenase (12), and an anionic peroxidase (5). Cloning of these types of pathway-controlling genes from *Cucurbita* has been greatly facilitated by the availability of probes and sequence information from other species where genes with the same function have already been cloned.

It is apparent from the literature that molecular genetics is just beginning in *Cucurbita*. As the genome is mapped, it should be possible to identify markers for many useful traits. Markers could be particularly useful for tagging the complementary virus-resistance genes, such as *Zym-2* and *Zym-3* (21) and ensuring that they are transferred during backcrossing. Molecular maps will also allow further investigation into the evolution and species relationships in *Cucurbita*, and between *Cucurbita* and other cucurbits.

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RAPD Markers Linked to Morphological and Disease Resistance Traits in Squash

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Molecular markers linked to phenotypic traits can be useful to breeders. The most useful types of markers are those which allow for selection in seedlings instead of mature plants, or which permit selection for disease resistance without inoculating the entire segregating population. Sometimes markers are developed for a specific trait of interest using bulked segregant analysis or near-isogenic lines. However, markers linked to morphological and disease resistance traits are often detected while constructing genetic maps. Saturated or nearly saturated genetic maps exist for a number of vegetable species, including cucumber and melon. *Cucurbita* has not been extensively mapped; the map published by Lee et al. (2) contained only five linkage groups and no morphological traits. The Random Amplified Polymorphic DNA (RAPD) marker – trait linkages reported here are part of a larger RAPD map of an interspecific *Cucurbita pepo* x *C. moschata* cross. The entire map will be published elsewhere.

RAPD markers are useful for making genetic maps in minor crops, as they are much less costly than Restriction Fragment Length Polymorphisms (RFLPs) or Simple Sequence Repeats (SSRs). However, they do have some recognized drawbacks. They are dominant markers. Reproducibility can be poor, particularly of complex banding patterns. Perhaps most significantly, RAPD markers cannot be readily transferred between populations, as they are identified by size rather than sequence. Thus RAPDs are most useful for marker assisted selection when they are tightly linked to a disease resistance gene or other trait which has been transferred from a single donor line into many different populations. Many of the difficulties with the reproducibility of markers can be solved by sequencing the RAPD band and creating a longer, more specific primer that identifies a Sequence Characterized Amplified Region (SCAR).

Material and Methods: *Plant material.* The mapping population consisted of 162 BC_1 individuals from a cross between a commercial yellow straightneck (*C. pepo*) inbred and *C. moschata* 'Nigerian Local'. The yellow squash was the recurrent parent. 'Nigerian Local' is resistant to zucchini yellow mosaic virus (ZYMV) and has been much used as a source of resistance in summer squash. The yellow squash inbred is resistant to powdery mildew. Morphological traits included precocious yellow fruit (the *B* gene), fruit flesh color, the intensity of fruit color, warts, mottled leaves, fruit shape, and spiny stems. The population segregated for resistance to the two diseases and for the morphological traits described above.

Assessment of Phenotype. Plants in the mapping population and five individuals from each parental line were grown to maturity in the greenhouse in Corvallis, Oregon from September through January, 1999-2000. All morphological data were collected using simple observation. Powdery mildew resistance was determined based on the severity of symptoms after extensive natural infection. Attempts were made to control the mildew only on the 'Nigerian Local' plants, which were known to be extremely susceptible. Virus inoculation with ZYMV was done on mature plants using a high-pressure paint sprayer to apply the inoculum directly to the growing points. We inoculated mature plants so that the virus symptoms would not interfere with data collection on the other morphological traits. One month after inoculation plants were visually rated for virus symptoms and tissue was collected for Enzyme Linked Immunosorbant Assay (ELISA) testing.

DNA Extraction and RAPD Analysis. DNA was extracted from newly expanded true leaves using a CTAB –chloform:isoamyl method (1). Following extraction the DNA was further purified by running each sample through a spin column re-precipitating the DNA (Genemate PCR Pure kit). This was done to remove salts and other compounds that were interfering with the Taq polymerase; it permitted us to reduce the amount of Taq in the reaction by 50%. DNA was quantified with a DyNAQuant fluorometer (Hoechst) and working solutions were standardized at 5 ng/ul DNA.

Parents were screened with 378 10-mer primers from Operon and the University of British Columbia.

Those primers that had strong bands present in Nigerian Local but not in the yellow squash were used to amplify the mapping population. Because the mapping population was the BC_1 to the yellow squash parent, bands present in that parent did not segregate in the progeny.

Reaction mixtures contained 30 ng of plant genomic DNA, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 3 pmole of primer (Operon), 0.5 units of Taq DNA polymerase (Promega), and 1X Taq buffer in a total volume of 15 ul. The amplification program was 2 min. at 94°C followed by 5 cycles of 5 seconds at 94°C, 1 min. at 37°C, 30 sec. at 54°C, a 7 min. ramp to 72°C, and 2 min. at 72°C and 30 cycles of 5 seconds at 94°C, 1 min. at 37°C, 30 sec. at 54°C, 2 min. at 72°C ending with 15 min. at 72°C. Because of the size of the population (162 BC1 individuals, 5 samples from each parental line, and parental and H_20 controls, for a total of 178 samples) it was necessary to amplify the population in two batches for each primer. Parental and H_2O controls were included in each batch, to enable us to detect any lack of repeatability between batches. In the interest of speed, four thermocyclers (an MJR-100, a Perkin-Elmer 9600 and two Perkin-Elmer 9700s) were used, but both batches for each primer were run on the same machine. In addition, the amplification programs were adjusted to be as identical as possible on the four machines. The PCR products were separated on 1.5% agarose (Seakem LE from FMC or Ultrapure from Gibco) in 0.5% TBE buffer. Size was determined with a 100-bp ladder (Promega). The gels were stained with ethidium bromide and recorded on Polaroid 667 film. Only strong, repeatable bands were scored.

Results: The yellow squash parent had smooth, intense yellow preanthesis fruit, which matured golden yellow. Peduncles were yellow and the fruit flesh was white. Leaves were uniform green. It was resistant to powdery mildew, but susceptible to ZYMV. The Nigerian Local parent had warty, intense black-green fruit and white flesh. Other plants in the population had white fruit or mottled dark- and light-green fruit. The leaves were mottled with silver. It was resistant to ZYMV but highly susceptible to powdery mildew. The F_1 plant had warty fruit, which was bicolor for intense yellow/intense green, and a green peduncle. The BC_1 progeny segregated for bicolor, peduncle color, color intensity, orange or white flesh, wartiness, mottled leaves, powdery mildew susceptibility and ZYMV susceptibility.

The morphological traits associated with markers were two affecting fruit color (including the economically important precocious yellow *B* gene), mottled leaf, and a virus resistance gene complementary to ZYMV resistance (*Zym*). RAPD markers are identified by the primer and the size of the band in base pairs. Map units are Kosambi centimorgans.

The precocious yellow gene (*B*) from the yellow squash parent was linked in repulsion to Operon $I10_{1700}$ at 27.1 cM. This linkage is somewhat questionable both because it is loose, and because $I10_{1700}$ and the RAPD markers most closely linked to it show distorted segregation, while *B* segregated 1:1 as expected. Operon $H14_{600}$ and UBC 489_{1200} flanked the dominant mottled leaf allele (*M*) from Nigerian Local. $H14_{600}$ is 13 cM from *M*, and 489_{1200} is 16.3 cM away. The actual linkages may be tighter, as the mottle-leaf trait was under-expressed in our low-light greenhouse. A gene controlling the intensity of mature fruit color is flanked by Operon $B14_{750}$ 17.8 cM away on one side and Operon $G17_{700}$ 9.7 cM away on the other. Both markers are linked to the fruit color gene in repulsion. A complementary gene for ZYMV resistance is 26.7 cM from Operon $L14_{1050}$. They are linked in coupling. The primary resistance gene *Zym* is epistatic to this gene, whose phenotype is a positive ELISA score for a symptomless plant one month after inoculation with ZYMV. We are currently conducting progeny tests to further understand this new gene. Allelism tests have not been done to determine its relationship to *Zym-2* and *Zym-3* (3). Other genes which have been mapped but remain unlinked are powdery mildew resistance (*pm*), orange fruit flesh, *Ep*, *Zym*, and warts (*Wt*).

Discussion: These five marked loci are only a beginning. They are not likely to be of great use in marker assisted selection because of the poor transferability of RAPDs, linkages in repulsion to the traits in *C. pepo*, and loose linkages. However, we hope to add additional markers to the squash map, including SSR markers, which are highly transferable. Progeny testing currently under way will tell us more about the new ZYMV resistance locus, and bulked segregant analysis may let us find markers which are more tightly linked. These could

be converted to SCARs for use in marker-assisted selection, as could the markers linked to leaf mottle.

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Genetic Variability in Pumpkin (*Cucurbita maxima*) Using RAPD Markers

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Introduction: Spain is one of the main producers in Europe of the gourd and pumpkin species *Lagenaria siceraria* (Mol.) Standl., *Cucurbita pepo* L., *Cucurbita moschata* Duchense, *Cucurbita ficifolia* Bouché and *Cucurbita maxima* Duchense. The Centre for Conservation and Breeding of Agricultural Biodiversity (COMAV) holds a collection of more than 900 accessions of these 5 species (4). A large number of these accessions belongs to *C. maxima*, collected from almost all the Spanish provinces. Most of them are traditional landraces adapted to diverse agro-ecological conditions, from mountainous dry lands to plain irrigated areas.

Since *C. maxima* is partially allogamous, a great deal of heterogeneity is expected within landraces. However, the number of plants included in traditional Spanish gardens is usually low. This small effective population size could influence the structure of the variation. Determining the degree of variability within an accession is necessary as a preliminary step for studying the genetic diversity among pumpkin accessions. In addition to the morphological characterization, a molecular screening is essential for determining this variability. RAPD markers are a fast and reliable method for this purpose.

In this work the polymorphism within and among different accessions of *C. maxima* is analysed by RAPDs. The convenience of using bulks of plants for further molecular studies is also evaluated.

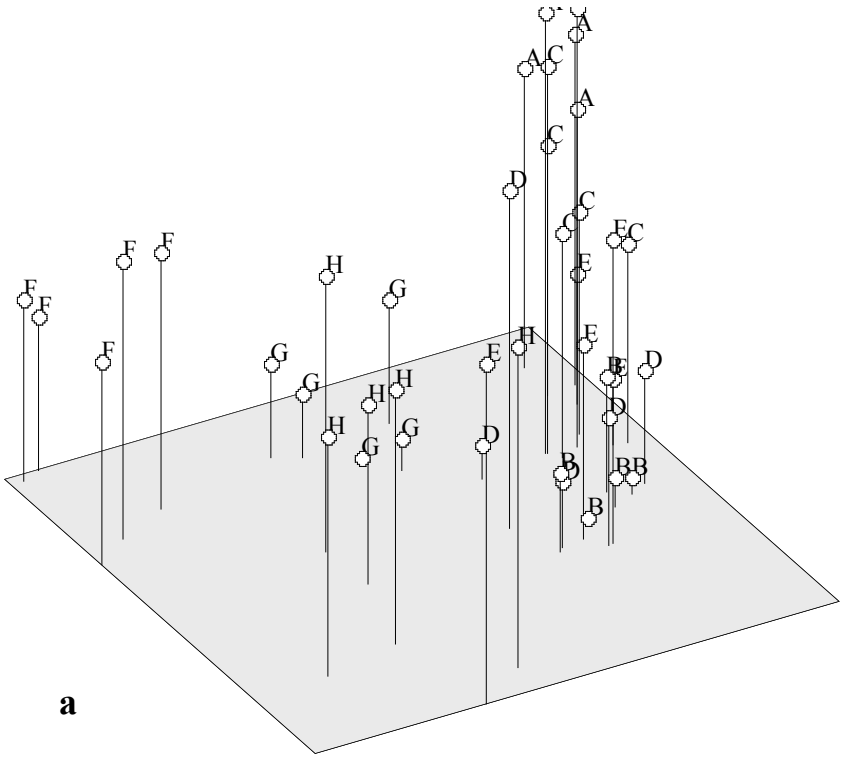
Materials and Methods: *Materials*: 8 accessions selected from a previous morphological characterization, following the Descriptor for *Cucurbita* of the IPGRI (3), were used. These accessions displayed different levels of variability concerning fruit morphological traits. The accessions CM, V1, V2 and AN2 are very uniform, with a granular skin texture, grey colour and variable ribs. All are used for human consumption. The accessions AS, CL1 and CL2, used for cattle feeding, are less variable. The differences among them concerns basically the colour and the shape of the fruit. Finally, the accession AN1, used for human consumption, shows the highest morphological variation.

Molecular characterization: DNA was extracted from leaf tissue of 5 plants per accession and from the bulk of these 5 plants using the method described by Doyle and Doyle (2). Single arbitrary 10-base primers selected from those described for pumpkin and other Cucurbitaceae species were used (6). Each 25µl PCR reaction mixture consisted of 50 ng genomic DNA, 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.3 µM primer, 10 x Taq buffer and 1 unit of Taq polymerase (Boehringer). The thermal profile for amplification was: 5 min of denaturing at 94ºC, 50 cycles with three steps each one: 1 min of denaturing at 94ºC, 1 min of annealing at 35ºC, and 2 min of elongation at 72ºC, with a final elongation step of 5 min at 72ºC. Visualization of amplification fragments was accomplished on a 2% agarose gel in 1 x TBE buffer stained with ethidium bromide.

Statistical analysis: Data scored as the presence (1) or absence (0) of amplification fragments were used to calculate Jaccard´s coefficients of similarity expressed as Euclidean genetic distances. The distance matrix was used to perform a principal coordinates analysis and a dendrogram.

Results and discussion: The 11 primers used in this study provided a total of 43 bands. 32 of them were polymorphic (74% polymorphism). The average distance between the different plants within accession was 0.3699 ± 0.988, ranging from 0.2662 ± 0.098 (accession V2) to 0.4288 ± 0.071 (accession CL2). The ANOVA and the Duncan mean comparison test indicated that the distances within the accession V2 were significantly smaller than those within the accessions AN1, AS, CL1 and CL2. An intermediate group included the accessions V1, AN2 and CM.

The principal coordinates analysis (Figure 1) shows how, in some cases, the plants of a particular accession are grouped jointly (V1, CM, AN2, V2, AS), while in other cases they are randomly distributed (AN1, CL1, CL2). This grouping and



A: CM, B: V1, C: V2, D: AN1, E: CL2, F: AN2, G: AS, H: CL1.

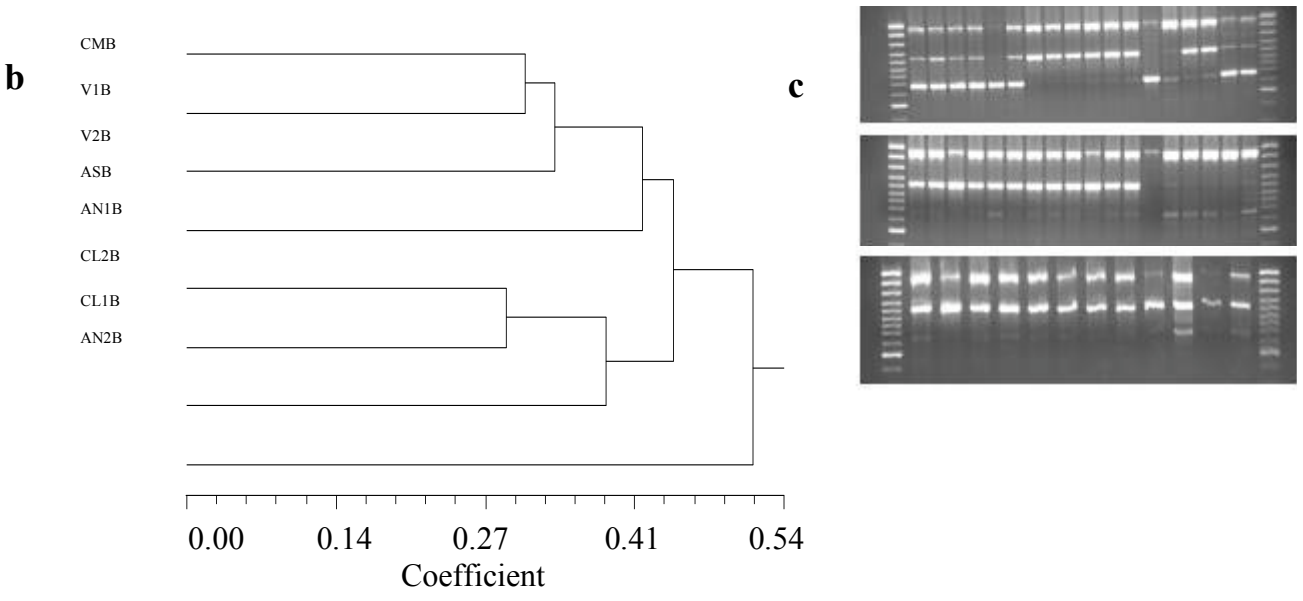


Figure 1. Variability within accessions in *C. maxima* (a): Principal coordinates analysis including 5 plants per accession, (b): Clustering of accessions using distances from bulking analysis, (c): Polymorphism within and among accessions using RAPDs. Each accession is represented by 5 plants and a bulk of these 5 plants.

those obtained by the ANOVA and the morphological characterization show a clear correspondence.

The heterogeneity of the analysed landraces involves a great genetic richness and increases the possibility of selection within them. However, this variability makes the analysis of the molecular diversity among accessions more difficult. For this type of study, the analysis of a great number of individuals per accession is the more accurate method, since even the alleles with low frequency in the population would be represented. However, it is very expensive and tedious for routine studies. The use of bulks of leaf tissue or DNA of several plants per accession could be more suitable, although it is less informative since only the most frequent alleles of the population are represented.

In this study, the average distance between accessions using bulks was 0.4328 ± 0.078, greater than that obtained within accessions. The dendrogram constructed from the bulks revealed three clusters that corresponded partially with the grouping based on fruit morphological characters (Figure 1). The usefulness of bulks for studying the variability among accessions is confirmed by the high correlation obtained between the distances among accessions calculated using bulks and those calculated using individual plants ($r = 0.732$; $P = 0.002$). Bulk analysis has also been reported in other allogamous species, such as *Brassica oleracea* L. (1) and *Lolium perenne* L. (5).

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Improving Culture Efficiency of *Cucumis metuliferus* Protoplasts

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Although we have made great progress in developing resistance to root – knot nematodes in cucumber, no source has been created or screened to resistance to *M. incognita,* the main species affecting cucumber (9, 10). Resistance to *M. incognita* exists in horned cucumber (*Cucumis metuliferus*), but efforts by many research groups (including our own) to make crosses of *Cucumis* species with cucumber have failed. Somatic hybridization using protoplast fusion is an alternative for utilizing *C. metuliferus* germplasm. Plants have been regenerated from protoplasts isolated from *C. sativus* (2, 4, 7), but not from *C. metuliferus.* To apply protoplast fusion, a reliable technique for isolating and culturing *C. metuliferus* protoplasts is often a prerequisite. Several reports suggest that large improvements in plating efficiency can be made by culturing protoplasts in a medium solidified with agarose (2, 3, 8).

The objective of this study was to compare agarose-disc culture to liquid culture, with and without weak light, for stimulating protoplast division of *C. metuliferus* cotyledon protoplasts. We also evaluated the efficiency of agarose-disc culture using different species (*C. sativus* and *C. metuliferus*), and protoplast sources (cotyledon or true leaf). Plating density, using nurse cultures, and center disc size (10 or 50 µl) were varied as well.

Methods. Seeds of two cultigens of cucumber ('Sumter' and Wisconsin SMR 18) and three plant introduction accessions of *C. metuliferus*, PI 482452, PI 482454 and PI 482461 were used. Sterilized seedlings were prepared as described as previous report with C1 medium (6). The protocols of protoplast isolation and mixture of enzyme solution with C2 medium were also same as previous report (6). Number of protoplasts was estimated using a hemacytometer after isolation, and viability was determined using fluroscein diacetate (11).

Influence of light and culture condition Only *C. metuliferus* PI 482454 were used in this experiment. For agarose culture, 10 ml of C2 medium with 1.2% (w/v) agarose (Sea Plaque FMC BioProducts, Rockland, Maryland) was used. Protoplasts were plated in agarose for a final density of 1×10^5 protoplasts per ml. Five 100 µl drops each of this solution were pipetted into 10 × 60 mm petri plates as described by Dons and Bouwer (3). After gelling, 4.5 ml of liquid C2 media was added. One and two weeks after isolation, the liquid medium was removed and C2 medium containing 0.275 or 0.25 M mannitol, respectively, was added. Agarose-disc cultures were maintained on a gyratory shaker at 30 rpm (3) at 30°C, under a 16 h photoperiod of cool white fluorescent lights (13.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (chamber 1), or in the dark at 30°C (chamber 2).

For liquid culture, protoplasts were cultured in 5 ml of C2 medium at a density of 1×10^5 protoplasts per ml in 10 × 60 mm petri plates and were maintained under the same conditions as the agarose-disc cultures, except keeping in a stationary position. Seven days after protoplast release, 1 ml of C2 medium, with only 0.15 M mannitol was added to each plate. Liquid culture plates were swirled one min per day to increase aeration. Estimates of protoplast division (PD), measured as the percentage of protoplasts which had undergone one cell division, and more than one division were made four and eight days after isolation. Cell wall regeneration was determined by observed changes in protoplast shape, and actual counts of cell division. The experiment was a split-block treatment arrangement in a randomized complete block design with three replications. There were three plates per treatment. Chambers were designated as whole plots, flasks were subplots, and culture techniques were sub-subplots.

Influence of genotype and tissue source. Two cultigens of cucumber ('Sumter' and Wisconsin SMR 18) and three accessions of *C. metuliferus*, PI 482452, PI 482454 and PI 482461 were used in this experiment. Protoplasts were isolated from cotyledons from five- to seven-day-old plants and leaves from 12- to 14-day-old plants. Cotyledon protoplasts were isolated from all five cultigens, while leaf protoplasts were isolated only from Wisconsin SMR 18 and PI 482454. In each replication, there was one flask per tissue type, with each flask contributing two plates to each treatment combination. To allow for adequate sampling, the lowest density, 10 µl disc treatments had three plates.

Agarose discs were prepared as described above, except that the final protoplast density of the center discs was reduced and nurse-culture discs were added to each plate. For each plate, four 100-µl nurse culture discs at a density of 1×10^5 protoplasts per ml were pipetted into the plate. A 10- or 50-µl disc of an estimated density of 5×10^2, 1×10^3, or 1×10^4 protoplasts per ml was then pipetted into the center of each plate. These center discs were used for evaluation of PD and plating efficiency (PE). Estimates of PD and cell wall regeneration were made four and eight days after isolation. PD were measured as described above. PE were estimated with the disc (s) by calculating the percentage of protoplasts which produced microcalli of 16 cells or more after 21 days. The experiment was a split-split plot treatment arrangement in a randomized complete block design with three replications. Mean comparisons for variables were made among treatments using Fisher's protected LSD (5% level).

Results. A wide range of concentrations of NaOCl (0.26 to 2.6%) were used to sterilize seeds of *C. metuliferus*, resulting in extremely low germination (0 to 5%). Use of the industrial disinfectant LD resulted in excellent germination rates (approximately 80%), and seed contamination of only 4 to 8% on C1 media (data not presented).

Influence of light and culture conditions. The number of viable protoplasts isolated per g of *C. metuliferus* PI482454 cotyledon tissue was $(1.25 \pm 0.22) \times 10^7$. Protoplast size varied from 10 to 50 µm in diameter. Cell wall regeneration and protoplast division began two to three days after isolation, regardless of treatment. After four days of culture, a significant difference in amounts of divided once (PD4-1) and more than once (PD4-2) protoplasts existed between agarose and liquid culture. No effect due to presence or absence of light was observed for PD4-1 and PD4-2 in either liquid or agarose culture (Table 1). Eight days after isolation, the percentage of divided once (PD8-1) and more than once protoplasts (PD8-2) was measured again, and significant differences between agarose and liquid culture still existed (Table 1). At that time, the presence of light resulted in significant increases for multiple protoplast divisions (PD8-2) only within agarose culture, but not in liquid culture.

The positive effect of using agarose medium on increasing both the number of protoplasts that had divided once and more than once support findings of numerous other researchers (1, 2, 5, 8) concerning the benefits of culturing protoplasts in agarose. The positive influence of weak light on multiple protoplasts division was only seen in agarose culture after eight days. Data from this experiment suggests that weak light is neither necessary or inhibitory for division.

Genotype and tissue source study. Protoplasts from cotyledon tissue varied in size from 10 to 50 µm in diameter. Primary leaf protoplasts for both species were more uniform in size, and varied in diameter from 10 to 20 µm in diameter. Yields and viability of protoplasts varied according to species and tissue type; all yielded acceptable quantities of viable protoplasts (Table 2).

After four days of culture, determination of multiple protoplast division (PD4) indicated a strong trend toward increased division with increased density was seen for both disc size treatments, and a more significant change was seen between 10 µl and 50 µl disc size treatments with culture densities of 1×10^3 and 1×10^4 protoplasts per ml (Table 3). A slower response of protoplasts in 10 µl disc treatments at a density of 1×10^3 protoplasts per ml was observed (Table 3).

Comparing different density treatment, significant effects due to density could also be seen within cotyledon protoplasts of both cucumber cultivars and one accession of *C. metuliferus* [(PI 482454 (Table 4). Increased density consistently caused an increase in multiple protoplast division. The percentage of protoplast division of 1×10^4 protoplasts per ml was higher than those of the other low densities after 4 to 8 day culture. However, disc size appears to have little long-term effect on multiple protoplast division and callus formation. After 21 days, plating efficiencies that was calculated as the percentage of protoplasts to form microcalli of 16 cells or more between 1×10^3 and 1×10^4 density treatments are very close, but higher than that of low density 5×10^2 protoplasts per ml (Table 4).

Table 1. Influence of light and culture conditions on *Cucumis metuliferus* protoplasts division.[z]

Culture conditions	Light ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Chamber	Day 4 PD4-1[y]	Day 4 PD4-2[x]	Day 8 PD8-1[y]	Day 8 PD8-2[x]
Agarose	13.5	1	27	3.1	25	29
	-	2	27	2.2	37	21
Liquid	13.5	1	7	0.0	17	3
	-	2	7	0.1	12	2
LSD (5%)			4	1.3	6	6
CV (%)			13	51	17	27

[z,y] Percentages of protoplasts which divided once and more than once.

Table 2. Protoplast isolation yields of different genotype and tissues.[z]

Genotype	Tissue source	Protoplast yield ($x10^6$/g)[y]	Protoplast viability (%)
Cucumis metuliferus			
PI 482452	Cotyledon	4.1 ± 1.5[x]	66.3 ± 30.6[x]
PI 482461	Cotyledon	7.9 ± 0.4	73.4 ± 17.0
PI 482454	Cotyledon	7.5 ± 2.3	77.9 ± 16.3
PI 482454	Leaf	13.0 ± 2.0	69.2 ± 8.4
Cucumis sativus			
Sumter	Cotyledon	2.4 ± 0.5	63.7 ± 19.1
Wis. SMR 18	Cotyledon	9.1 ± 0.5	78.6 ± 8.1
Wis. SMR 18	Leaf	3.5 ± 1.1	62.3 ± 8.9

[z] Data are means of 3 replications.

[y] Viable protoplasts released/g fresh weight of material.

Table 3. Effect of center disc size and low protoplast densities on the percent multiple protoplast division 4 days after isolation (%).[z]

Disc size	Protoplast density (no./ml agarose) $5x10^2$	$1x10^3$	$1x10^4$
10 µl	0.4	1.1	3.9
50 µl	0.4	3.0	4.2

[z] Data are means of 7 combinations of genotypes and tissue source (see table 2). Each combination has 3 replications, 3 samples and 2 subsamples.

Table 4. Effect of different densities with nurse cultures on the multiple protoplast division and plating efficiency in *Cucumis metuliferus* and *C. sativus*.[z]

Protoplast density (no./ml agarose)	Protoplast division(%) Day 4	Day 8	Plating efficiency(%) Day 21
$5x10^2$	0.4	5.0	2.2
$1x10^3$	2.0	10.9	4.2
$1x10^4$	4.1	15.9	5.1
LSD (5% for column comparisons)	2.1	4.6	2.5

[z] Data are means of 14 treatments (7 genotype and tissue source combination with 2 disc sizes: 10 and 50 µl). Each treatment has 3 replications, 3 samples and 2 subsamples.

[y] Means with different letters are significantly different at 5%.

Table 5. Effect of different tissue source with nurse cultures on the multiple protoplast division and plating efficiency in *Cucumis metuliferus* and *C. sativus*.[z]

Protoplast source	Protoplast division(%) Day 4	Day 8	Plating efficiency(%) Day 21
Cotyledon	2.9a[y]	13.6a	5.2a
Leaf	0.0b	3.0b	0.1b

[z] Data of leaf protoplast are means of 2 genotypes with two disc sizes and data of cotyledon protoplast are means of 5 genotypes with two disc size. Each treatment has 3 replications, 3 samples and 2 subsamples.

[y] Means with different letters are significantly different at 5%.

Moreover, great differences for multiple protoplast division were also seen between cotyledon and leaf protoplasts (Table 5). Generally leaf protoplasts that had not lysed by day four showed little or no cell-wall development or cell division for the duration of the experiment. Although an acceptable yield of protoplasts was obtained from all species and tissue types (Table 2), the culture technique used was not suitable for leaf protoplasts (Table 5). The current isolation technique or some aspect of the culture media could be the cause of the poor survival and division rate of leaf protoplasts although leaf protoplasts of *C. sativus* have been successfully cultured using agarose (2, 7).

Throughout this study, the densities between 1×10^3 and 1×10^4 protoplasts per ml with agarose nurse culture is an efficient technique for both *C. metuliferus* and *C sativus* protoplast isolation and culture. The importance of weekly replenishment of limiting nutrients and plant-growth regulators should not be ignored as a possible advantage of agarose over liquid culture. However, despite trials using many different media and growth regulator combinations, no shoot differentiation from the callus of either species has occurred to date. The regeneration technique need to be further studied.

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Isolation and Callus Production from Cotyledon Protoplasts of *Cucumis metuliferus*

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In North Carolina, approximately 8% of the cucumber (*Cucumis sativus*) yield is lost to root-knot nematodes (*Meloidogyne* spp.) (Main and Gurtz, 1989). No resistance was found in cucumber after screening of 900 cultigens for resistance to *Meloidogyne incognita* race 3 (Walters, 1991). African horned cucumber (*Cucumis metuliferus*) has resistance to *M. incognita* (Fassuliotis, 1967; Walters, 1991), as well as other diseases such as squash mosaic virus and watermelon mosaic virus (Provvidenti and Robinson, 1974). There would be benefit in transferring nematode resistance into the gene pool of *C. sativus* from *C. metuliferus*. However, traditional sexual hybridization techniques have been unsuccessful in producing hybrids between *C. metuliferus* and cucumber, probably due to differences in chromosome number (Deakin et al., 1971) or incompatibility barriers. In some crops, use of protoplast fusion has allowed barriers to sexual hybridization to be overcome.

Within *Cucumis*, most protoplast isolation and plant regeneration studies have been attempted using *C. sativus* and *Cucumis melo* (Wehner et al., 1990). Plant regeneration from protoplasts of *C. sativus* was first reported by Jia et al. (1986). The successful protocol for asymmetric protoplast fusions of cucumber and melon has been developed (Jarl et al., 1995). However, there were few reports involving protoplast isolation and fusion with *C. metuliferus*. In one study, a fusion rate of 5 to 12% was reported between protoplasts of *C. metuliferus* and *C. melo*. Their attempts to obtain callus from protoplasts of *C. metuliferus* or from fusion products were unsuccessful (Roig et al., 1986). In a second study, Tang and Punja (1989) reported protoplast fusion of *C. metuliferus* and *C. sativus,* and subsequent callus development from hybrid protoplasts. However, they were unable to produce callus from protoplasts of *C. metuliferus* alone, or to regenerate plants from callus of hybrid protoplast.

Development of an efficient system of plant regeneration from *C. metuliferus* protoplasts may assist in obtaining a procedure to regenerate hybrid plants from protoplast fusion of *C. sativus* and *C. metuliferus*. Therefore, the objective of this study was to develop a reliable procedure for isolation and culture of cotyledon protoplasts of *C. metuliferus*.

Methods. Seeds of *C. metuliferus* PI 482454 were surface-sterilized with the industrial disinfectant LD (Alcide Corp., Norwalk, Conn.) for 30 minutes on a gyratory shaker at 100 RPM, then were rinsed 5 times with sterilized, distilled water. Seeds were germinated on C1 medium (Table 1) in the dark at 30°C. After 84 hr, seedlings were placed in a culture room maintained at 22°C with a 16 hr photoperiod lights (108 $\mu Mol \cdot m^{-2} \cdot s^{-1}$). Seedlings were transferred back to 30°C in darkness for 24 hours before protoplast isolation.

An enzyme solution was prepared consisting of 0.7 mM KH_2PO_4, 7 mM $CaCl_2 \cdot 2H_2O$, 0.5 M mannitol, 3 mM MES [2-(N-morpholino) ethanesulfonic acid] (Jia et al., 1986), 2 % (w/v) cellulysin and 0.5 % (w/v) macerase (Calbiochem, La Jolla, California). That solution was mixed at a 1:1 (v/v) ratio with C2 medium having a pH of 5.8 (Table 1), except modified by the addition of 230 $mg \cdot L^{-1}$ $CaCl_2 \cdot 2H_2O$ as described by Jia et al. (1986). Ten ml of filter-sterilized enzyme C2 solution was added to 0.5 g of whole cotyledons (5 to 7 days old), and vacuum infiltrated at 9.3 kPa for 20 sec. The infiltrated tissue was incubated for 6 hr on a gyratory shaker at 60 rpm at 25°C in the dark. Protoplasts were separated from cell walls and other debris by manually swirling the flasks, and filtering the solution through sterilized Miracloth (Calbiochem, La Jolla, California).

Protoplasts were washed 3 times with C2 medium by centrifuging at 100x g for 3 minutes. For all experiments, protoplast density after isolation was estimated using a hemacytometer, and viability was determined using fluroscein diacetate (Widholm, 1972). Protoplasts were cultured in 5 ml of C2 medium at a density of $1x10^5$ protoplasts per ml in 10 x 60 mm petri plates, and incubated in the dark at 25°C. Five days after protoplast isolation, half of the

plates were transferred to a growth chamber maintained at 30°C in the dark. Seven days after protoplast release, 1 ml of C2 medium with 0.15 M mannitol was added to each plate. Fourteen and 21 days after isolation, 1 ml of C2 medium (with no mannitol) was added to each plate. Protoplast culture plates were swirled 1 min per day to increase aeration.

Estimates of protoplast division (PD), calculated as the percentage of protoplasts which had undergone cell division, were made 8 to 10 days after isolation by visual observation of 5 samples per plate with 15 plates per treatment combination (320x magnification). Cell wall regeneration was determined by observed changes in protoplast shape and actual cell division. Using the sample results, total number of divided cells per plate was calculated. This number was then compared to the total number of protoplasts in the plate (5×10^5) to produce an estimate of PD. Approximately 3 weeks after isolation, plating efficiency (PE) was estimated by counting clumps of 8 or more cells which appeared to have originated from 1 protoplast. PE was estimated by counting the number of microcalli in 5 samples per plate with 15 plates per replication (100x magnification), and calculating an approximate number per plate from the random visual counting. Experiment 1 was a randomized complete block design with 4 replications. Treatments consisted of protoplasts cultured in C2 medium at either 25 or 30°C.

In experiment 2, protoplasts were isolated and cultured in C2 medium at either 25 or 30°C, using the methods described above. After 3 weeks, 2 ml of microcallus suspension were pipetted onto C4 medium (Table 1) (20 ml in 100 x 15 mm petri dishes) containing varying amounts of 2,4-D, 1H-indo-3-acetic acid (IAA), kinetin and N-(phenylmethyl)-1H-purine-6-amine (BA) (Table 2). Plates were swirled to distribute microcalli on the agar medium, and excess liquid medium was removed. Callus cultures were maintained at 22°C in the dark for 3 weeks before being rated for percentage of each petri plate covered with callus. Callus color was rated 1 to 9 (1=white, 5=yellow, 9=brown). After rating callus, clumps of healthy (white to yellow in color) callus were transferred to similar media, and to C4 media containing 0.2 $mg \cdot L^{-1}$ IAA and 5.0 $mg \cdot L^{-1}$ kinetin (Jia et al., 1986). Experiment 2 was a randomized complete block design with 4 plates per treatment and 4 replications. Treatments consisted of protoplasts that had been first cultured in C2 medium at 25 or 30°C for 3 weeks cultured on C4 medium containing varying amounts of growth regulators at 22°C (Table 2).

In experiment 3, protoplasts were isolated and cultured in C2 medium at either 25 or 30°C, using the methods described in experiment 1. After 3 weeks, protoplasts were transferred to C3 medium for callus induction. Treatments consisted of the same growth regulator combinations as used in experiment 2, using protoplasts which had been cultured at either 25 or 30°C for the first 3 weeks (Table 3). Callus cultures were maintained at 22°C in the dark for 6 weeks before rating each petri plate for percent coverage with callus. Experiment 3 was a randomized complete block design with 3 plates per treatment and 5 replications.

Results. In experiment 1, protoplasts cultured at 25°C had a protoplast division percentage (PD) of 4% after 8 to 10 days. Protoplasts cultured at 30°C had a PD of 7%. There was a significant difference between the 2 temperatures for PD. After 3 weeks of culture at 25°C, each plate had a mean of 3970 microcalli per plate (PE of 0.8%). At 30°C, each plate had a mean of 5025 (PE of 1.0%) microcalli per plate.

In experiment 2, medium A3 (Table 2) was best for producing a large amount of yellow, friable callus. There were no significant differences in color among media. However, among temperatures, protoplasts cultured at 30°C were significantly whiter (Table 2). Callus color appeared to indicate the potential for continued proliferation because callus with ratings >5 usually had little or no continued growth. Healthy callus transferred to C3 or C4 media containing 0.2 $mg \cdot L^{-1}$ IAA and 5.0 $mg \cdot L^{-1}$ kinetin caused sporadic greening. Five to 15 roots per plate were observed on callus on media A3 and A4 after 6 weeks. No embryogenesis resulting in plantlet regeneration was observed in this experiment.

Table 1. Components used for culture media for explants and protoplasts of *C. metuliferus*.[z]

Component	Culture medium tested C1	C2	C3	C4
Mannitol	-	0.3 M	-	-
2,4-D	-	0.5 mg/L	-	-
Kinetin	-	1.0 mg/L	-	-
Agar (w/v)	0.8 %	-	0.8 %	0.8 %
Salts	1/2 MS[y]	Mod DPD[x]	MS	Mod. DPD
Vitamins	1/2 MS	DPD	B5[w]	DPD
Sucrose ($g \cdot L^{-1}$)	15.0	17.1	30.0	17.1

[z] All media were adjusted to a pH of 5.8.

[y] Murashige and Skoog (1962).

[x] Durand et al. (1973) modified by Jia et al. (1986).

[w] Gamborg et al. (1968).

Table 2. Callus growth from protoplasts of *C. metuliferus* after 3 weeks on 4 different media, at 22°C in the dark.[z]

Medium no.	Media components[y]	Plate coverage(%) 25°C	30°C	Mean	Color rating 25°C	30°C	Mean
A1	0.01 mg 2,4-D + 1.0 mg BA	3.0	5.0	4.0	6.1	5.0	5.6
A2	0.20 mg IAA + 0.5 mg BA	0.0	3.0	1.0	-	5.5	5.5
A3	0.25 mg 2,4-D + 0.5 mg kin.	10.0	8.0	9.0	5.3	4.4	4.8
A4	0.50 mg 2,4-D + 1.0 mg kin.	2.0	5.0	3.0	5.3	3.7	4.1
	Mean (LSD 5%[x])	3.7	5.6	(NS)	5.6	4.5	(0.7)

[z] Data are means of 4 replications; protoplasts were initially cultured for 3 weeks at 25 or 30°C; initial medium was C2 (Table 1).

[y] Base medium was C4 (Table 1); all growth regulators expressed in $mg \cdot L^{-1}$.

[x] For comparison of column means.

Table 3. Callus growth from protoplasts of *C. metuliferus* after 6 weeks on 4 different media, at 22°C in the dark.[z]

Medium		Plate coverage(%)			Color rating		
no.	Media components[y]	25°C	30°C	Mean	25°C	30°C	Mean
B1	0.01 mg 2,4-D + 1.0 mg BA	3.0	6.9	5.9	3.0	6.0	5.0
B2	0.20 mg IAA + 0.5 mg BA	1.5	16.1	11.2	5.0	5.4	5.3
B3	0.25 mg 2,4-D + 0.5 mg kin.	0.0	5.0	2.5	-	4.0	4.0
B4	0.50 mg 2,4-D + 1.0 mg kin.	0.0	9.3	6.2	-	6.6	6.6
	Mean (LSD 5%[x])	1.0	10.3	(3.2)	4.0	5.6	(0.4)

[z] Data are means of 5 replications; protoplasts were initially cultured for 3 weeks at 25 or 30°C; initial medium was C2 (Table 1).

[y] Media B1 to B3 consist of C3 medium; medium B4 consists of C4 medium; all growth regulators expressed in $mg \cdot L^{-1}$.

[x] For comparison of column means.

In experiment 3, growth regulator concentrations remained the same, but the basal medium was changed from C4 to C3. In contrast to experiment 2, no significant differences were observed for media types affecting amount of callus. However, media type B3 produced whiter callus than other media (Table 3). There was significantly more growth at 30 than at 25°C, repeating the trend seen in experiments 1 and 2. Effects of temperature on color, however, were the opposite of results of experiment 2, with protoplasts cultured at 25°C being significantly whiter (Table 3). After 6 weeks on medium B3 or B4, more than 10 roots per plate were obtained. As with experiment 2, no embryogenesis was observed.

Results of all 3 experiments suggest that a culture temperature at or near 30°C produces more microcalli and/or callus. In experiment 3, callus from protoplasts cultured at 30°C was browner, perhaps reflecting a depletion of nutrients in the medium. This effect was probably due to increased growth at the higher temperature compared to 25°C, and can probably be corrected by more frequent subculturing.

In summary, a method has been developed which consistently provides large numbers of viable protoplasts from cotyledon tissue of *C. metuliferus*. The technique allows development of callus and roots from the protoplast cultures. Using our procedure, 30°C was better than 25°C for producing microcalli, and large amounts of callus on the callus induction media. Although there was a large amount of variability associated with liquid culture, agar solidified MS medium containing 0.25 $mg \cdot L^{-1}$ 2,4-D and 0.5 $mg \cdot L^{-1}$ kinetin was best suited for producing large amounts of friable, light yellow callus.

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Seed Treatment Effects on Emergence of Luffa Sponge Gourd

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Luffa sponge gourd (*Luffa aegyptiaca* Mill.; also *L. cylindrica*) belongs to the Cucurbitaceae along with squash and pumpkin (*Cucurbita* spp.), gourd (*Cucurbita* spp., *Lagenaria* spp.), melon (*Cucumis melo* L.), and cucumber (*Cucumis sativus* L.). It is an annual climbing vine with tendrils, growing primarily in tropical and subtropical regions. The seeds are flat, smooth, and black or white (11). This species has been cultivated since ancient times in the far east and the Indian subcontinent, and has since been introduced to the Middle-east and Africa, and more recently into the Americas. The fruit is harvested at an immature stage (both fruit and seeds are soft), and is eaten as a cooked vegetable (prepared like summer squash). In most countries, it is cultivated in small plots in family gardens, usually for domestic consumption, with commercial cultivation being of secondary importance. However, it is beginning to appear in the international market place (6, 7).

Germination percentages of several vegetable species have been shown to increase after seed treatment with chemicals and various osmotica (1, 4, 10, 13, 18). Luffa seed germination has been reported to be slow and sporadic (2). Low percentage of seed germination is a major problem in establishing a luffa crop, with typical rates of less than 75% (17).

The seeds of many species of cucurbits are non-endospermic and germination is epigeal. Dormancy can be severe problem in some cucurbit species. It is comparatively easy to induce dormancy by testing the seeds for germination in unfavorable environments. In particular, the germination test substrate should not be too moist and only very low intensity light treatments should be applied. In most cases, it is probably best to perform seed germination tests in darkness. Successful dormancy-breaking treatments in sponge gourd are observed to be use of constant temperatures with optimum range of 30 to 35°C, scarification of the seeds before germination, cracking the seed coat before germination, and use of moist sand for the germination medium.

In contrast to other members of the Cucurbitaceae, the germination of sponge gourd seeds is promoted by light (3). Poor field emergence and erratic stands lead to increased variation in plant development which can result in yield reduction. Both survival and performance of seeds after sowing are affected by physical, chemical, and biotic factors. Temperature, light, drought, flooding, and gaseous (O_2 and CO_2 concentration) environments are physical factors that influence seedling emergence (5, 8, 14). Poor field emergence is consistently observed where spring temperatures fluctuate dramatically (9, 15, 16). In areas where crops are harvested once-over (for example, cucumbers that are machine-harvested), non-uniform emergence is of particular concern (13). Since a species cannot become established in and colonize new habitat until it has completed the stages of germination and seedling establishment, it is most important to study the effect of soil factors on the germinating seeds and young seedlings (12).

There is little published information on the effects of seed treatment on germination of luffa gourd. It would be useful to increase the germination rate of luffa seeds, especially for those interested in commercial production of the crop. Therefore, the objective of this study was to determine if treating luffa sponge gourd seeds prior to planting would improve the germination rate.

Methods. This study was conducted in two parts. Experiment one consisted of a single cultivar (Fletcher) and nine seed pre-treatments. The design was a randomized complete block with five replications of ten seeds each. The seed pre-treatments were: acetone soaking (16 hours at 25°C), sandpaper (25 shakes in an 75 x 305 mm tube lined with coarse-grade sandpaper), soaking in a beaker of water (not aerated) for 12, 24, and 36 hours at 25°C, or moist cloth bags (102 x 152 mm cotton bags) soak for 12, 24, and 36 hours at 25°C. The moist cloth bags were wetted after seeds were added by soaking under water for 1 minute. Bags were kept moist by adding 10 ml of water every 24 hours. The control was untreated seeds. The experiment was started on 11 October. Seed pre-treatments were performed on

the preceding one or two days (depending on treatment duration).

Experiment two had three cultigens (Fletcher, PI 163295, and PI 391603) and eleven seed pre-treatments with four replications of ten seeds per replication. The treatments included all those of experiment one, along with soaking in a water beaker for 48 hours, and moist cloth bags soaked for 48 hours. The acetone treatment was reduced to 12 hours followed by three water rinses prior to seeding. The experiment was started on 2 November. Seed pre-treatments were performed on the preceding one or two days (depending on treatment duration).

In both experiments, the seeds were planted in a peat-lite mix in 100-mm diameter plastic pots with 10 seeds each. The pots were placed on heating mats set at 32.2°C for the duration of the experiment. Emergence data were collected daily for 12 days (15 days for experiment two). The data collected were analyzed using PROC GLM of SAS.

Results. The first experiment was conducted to evaluate the effects of seed treatments on emergence. The results revealed no significant differences among the seed treatments except the acetone treatment which was significantly lower (56%) than the other treatments (Table 1). The control emergence was higher than the mean suggesting there were no emergence problems associated with this lot of seeds.

In the second experiment, seeds with previously tested germination rates were used. 'Fletcher' initially had a germination rate of 62%, PI 163295 and PI 391603 each had a germination rate of 70%. The seed pre-treatment times were increased to determine whether germination could be improved. The analysis of variance (data not shown) indicated highly significant differences among cultigens and significant differences for the cultigen by treatment interaction. However, most seed pre-treatments were not significantly different from the control. 'Fletcher' seed germination was significantly better than that of PI 163295 and PI 391603. PI 163295 and PI 391603 were not significantly different from each other.

Table 1. Effects of seed treatments on percentage of emergence of luffa sponge gourd using three cultigens in two experiments.

Seed germination pre-treatment	Experiment 1	Experiment 2		
	Fletcher	Fletcher	PI 391603	PI 163295
Acetone (16 hr, no)	56	-	-	-
Acetone (12 hr, rinse)	-	85	32	28
Sandpaper	92	88	55	40
Water soak 12 hr	98	80	52	65
Water soak 24 hr	90	88	60	65
Water soak 36 hr	94	95	65	40
Water soak 48 hr	-	88	60	30
Moist cloth bag 12 hr	90	82	42	42
Moist cloth bag 24 hr	92	72	42	68
Moist cloth bag 36 hr	98	78	58	58
Moist cloth bag 48 hr	-	85	72	50
Control	96	95	58	42
LSD (5%)	12	7	7	7
Mean	90	85	54	48
CV (%)	2	2	2	2

[Z] Data are means of 5 replications of 10 seeds each for experiment 1, and 4 replications of 10 seeds each for experiment 2. Water soaking treatments did not include aeration.

A previous experiment using 'Fletcher' luffa gourd showed no improvement in seed germination after pre-treating the seeds with growth regulators such as gibberellic acid-3 and ethephon (17).

Based on the tests conducted, acetone is not recommended as a seed treatment for improvement of seed germination in luffa gourd. The remaining treatments could be used to pregerminate seeds so as to remove seeds that were slow germinators or non-germinators. Pregermination would also be useful to establish a crop in the field with one plant per hill, and with all plants at the same stage of growth.

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Gene List 2001 for Cucumber

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This is the latest version of the gene list for cucumber (*Cucumis sativus* L.). Complete lists and updates of genes for have been published previously (Pierce and Wehner, 1989; Robinson et al., 1976; Robinson et al., 1982; Wehner, 1993; Wehner and Staub, 1997). For the first time, this list includes genes that have been cloned from different plant tissues of cucumber. The genes on the 2001 list are of ten categories as follows: seedling markers, stem mutants, leaf mutants, flower mutants, fruit type mutants, fruit color mutants, resistance genes (mostly to diseases), protein (isozyme) variants, DNA (RFLPs and RAPDs) markers (Table 1), and cloned genes (Table 2).

Revisions to the 1997 cucumber gene list include the addition of nine genes that have been reported during past 5 years, including: *bi-2, mj, msm, Prsv-2, rc-2, wmv-2, wmv-3, wmv-4*, and *zym-Dina*. Six genes for virus resistance (*mwm, zym, Prsv-2, wmv-2, wmv-3*, and *wmv-4*) come from one inbred TMG-1.

Genes that have been published in previous lists but modified in this list are *zymv* (renamed *zym*, and then *zym-TMG1* to distinguish it from *zym-Dina*). The gene *mwm* published in the literature may be the same as *zym-TMG1*. We also corrected the symbol for the flower mutant, *male sterile-2 pollen sterile*, $ms\text{-}2^{(PS)}$ (Zhang et al., 1994), with the superscript in parentheses to indicate an indistinguishable allele.

Isozyme variant nomenclature for this gene list follows the form according to Staub et al. (Staub et al., 1985), such that loci coding for enzymes (e.g. glutamine dehydrogenase, G2DH) are designated as abbreviations, where the first letter is capitalized (e.g. G2dh). If an enzyme system is conditioned by multiple loci, then those are designated by hyphenated numbers, which are numbered from most cathodal to most anodal and enclosed in parentheses. The most common allele of any particular isozyme is designated 100, and all other alleles for that enzyme are assigned a value based on their mobility relative to that allele. For example, an allele at locus 1 of FDP (fructose diphosphatase) which has a mobility 4 mm less that of the most common allele would be assigned the designation *Fdp(1)*-96.

RFLP marker loci were identified as a result of digestion of cucumber DNA with *Dra*I, *Eco*RI, *Eco*RV, or *Hind*III (Kennard et al., 1994). Partial-genomic libraries were constructed using either *Pst*I-digested DNA from the cultivar Sable and from *Eco*RV-digested DNA from the inbred WI 2757. Derived clones were hybridized to genomic DNA and banding patterns were described for mapped and unlinked loci (CsC482/H3, CsP314/E1, and CsP344/E1, CsC477/H3, CsP300/E1).

Clones are designated herein as CsC = cDNA, CsP = *Pst*I-genomic, and CsE = *Eco*RI-genomic. Lower-case a or b represent two independently-segregating loci detected with one probe. Lower-case s denotes the slowest fragment digested out of the vector. Restriction enzymes designated as DI, *Dra*I; EI, *Eco*RI; E5, *Eco*RV; and H3, *Hind*III. Thus, a probe identified as CsC336b/E5 is derived from a cDNA library (from 'Sable') which was restricted using the enzyme *Eco*RV to produce a clone designated as 336 which displayed two independently segregating loci one of which is b. Clones are available in limited supply from Jack E. Staub.

RAPD marker loci were identified using primer sequences from Operon Technologies (OP; Alameda, California, U.S.A.) and the University of British Columbia (Vancouver, BC, Canada). Loci are identified by sequence origin (OP or BC), primer group letter (e.g., A), primer group array number (1-20), and locus (a, b, c, etc.) (Kennard et al., 1994). Information regarding unlinked loci can be obtained from Jack E. Staub.

Because of their abundance, common source (two mapping populations), and the accessibility of published information on their development (Kennard et al., 1994) DNA marker loci are not included in Table 1, but are listed below.

The 60 RFLP marker loci from mapping cross Gy 14 x PI 183967 (Kennard et al., 1994): CsP129/E1, CsC032a/E1, CsP064/E1, CsP357/H3, CsC386/E1, CsC365/E1, CsP046/E1, CsP347/H3, CsC694/E5, CsC588/H3, CsC230/E1, CsC593/D1, CsP193/H3, CsP078s/H3, CsC581/E5, CsE084/E1, CsC341/H3, CsP024/E1, CsP287/H3, CsC629/H3, CsP225s/E1, CsP303/H3, CsE051/H3, CsC366a/E5, CsC032b/E1, CsP056/H3, CsC378/E1, CsP406/E1, CsP460/E1, CsE060/E1, CsE103/E1, CsP019/E1, CsP168/D1, CsC560/H3, CsP005/E1, CsP440s/E1, CsP221/H3, CsC625/E1, CsP475s/E1, CsP211/E1, CsP215/H3, CsC613/E1, CsC029/H3, CsP130/E1, CsC443/H3, CsE120/H3, CsE031/H3, CsC366b/E5, CsC082/H13, CsP094/H3, CsC362/E1, CsP441/E1, CsP280/H3, CsC137/H3, CsC558/H3, CsP037a/E1, CsP476/H3, CsP308/E1, CsP105/E1, and Csc166/E1.

The 31 RFLP marker loci from mapping cross Gy 14 x PI 432860 (Kennard et al., 1994): CsC560/D1, CsP024/E5, CsP287/H3, CsC384/E5, CsC366/E5, CsC611/D1, CsP055/D1, CsC482/H3, CsP019/E1, CsP059/D1, CsP471s/H13, CsC332/E5, CsP056/H3, CsC308/E5, CsP073/E5, CsP215/H3, CsC613/D1, CsP266/D1, CsC443/H3, CsE031/E1, CsE120/H3, CsE063/E1, CsP444/E1, CsC612/D1, Cs362/E1, CsP280/H3, CsC558/H3, CsP008/D1, CsP308/E1, CsC166/E1, and CsP303/H3.

The 20 RAPD marker loci from mapping cross Gy 14 x PI 432860 (Kennard et al., 1994): OPR04, OPW16, OPS17, OPE13a, OPN06, OPN12, OPP18b, BC211b, OPN04, OPA10, OPE09, OPT18, OPA14b, OPU20, BC460a, OPAB06, OPAB05, OPH12, OPA14a, and BC211a.

In addition to the isozymes, RFLPs and RAPDs, nearly 100 cloned genes are listed here (Table 2).

Researchers are encouraged to send reports of new genes, as well as seed samples to the cucumber gene curator (Todd C. Wehner), or to the assistant curators (Jack E. Staub and Richard W. Robinson). Please inform us of omissions or errors in the gene list. Scientists should consult the list as well as the rules of gene nomenclature for the Cucurbitaceae (Robinson et al., 1976; Robinson et al., 1982) before choosing a gene name and symbol. That will avoid duplication of gene names and symbols. The rules of gene nomenclature were adopted in order to provide guidelines for naming and symbolizing genes. Scientists are urged to contact members of the gene list committee regarding rules and gene symbols.

Table 1. The non-molecular genes of cucumber.

Gene	Synonym	Character	References[z]	Supplemental references[z]	Available[y]
a	-	*androecious*. Produces primarily staminate flowers if recessive for *F*. *A* from MSU 713-5 and Gy 14; *a* from An-11 and An-314, two selections from 'E-e-szan' of China.	Kubicki, 1969		P
Ak-2	-	*Adenylate kinase* (E.C.# 2.7.4.3). Isozyme variant found segregating in PI 339247, and 271754; 2 alleles observed.	Meglic and Staub, 1996		P
Ak-3	-	*Adenylate kinase* (E.C.# 2.7.4.3). Isozyme variant found segregating in PI 113334, 183967, and 285603; 2 alleles observed.	Meglic and Staub, 1996		P
al	-	*albino cotyledons*. White cotyledons and slightly light green hypocotyl; dying before first true leaf stage. Wild type Al from 'Nishiki-suyo'; *al* from M_2 line from pollen irradiation.	Iida and Amano, 1990, 1991		?
ap	-	*apetalous*. Male-sterile. Anthers become sepal-like. *Ap* from 'Butcher's Disease Resisting'; *ap* from 'Butcher's Disease Resisting Mutant'.	Grimbly, 1980		L

Ar	-	*Anthracnose resistance*. One of several genes for resistance to *Colletotrichum lagenarium*. *Ar* from PI 175111, PI 175120, PI 179676, PI 183308, PI 183445; *ar* from 'Palmetto' and 'Santee'.	Barnes and Epps, 1952		P
B	-	*Black or brown spines*. Dominant to white spines on fruit.	Strong, 1931; Tkachenko, 1935; Wellington, 1913	Cochran, 1938; Fujieda and Akiya, 1962; Hutchins, 1940; Jenkins, 1946; Youngner, 1952	W
B-2	-	*Black spine-2*. Interacts with *B* to produce F_2 of 15 black: 1 white spine. *B-2* from Wis. 9362; *b-2* from PI 212233 and 'Pixie'.	Shanmugasundarum et al., 1971a		?
B-3	-	*Black spine-3*. Interacts with *B-4* to produce an F_2 of nine black: 7 white spine. *B-3* from LJ90430; *b-3* from MSU 41.	Cowen and Helsel, 1983		W
B-4	-	*Black spine-4*. Interacts conversely with *B-3*. *B-4* from LJ90430; *b-4* from MSU 41.	Cowen and Helsel, 1983		W
bi	-	*bitterfree*. All plant parts lacking cucurbitacins. Plants with *bi* less preferred by cucumber beetles. Plants with *Bi* resistant to spider mites in most American cultivars; *bi* in most Dutch cultivars.	Andeweg and DeBruyn, 1959	Cantliffe, 1972; Da Costa and Jones, 1971a, 1971b; Soans et al., 1973	W
bi-2		*bitterfree-2*. Leaves lacking cucurbitacins; *bi-2* from NCG-093 (short petiole mutant).	Wehner et al., 1998a		W
bl	*t*	*blind*. Terminal bud lacking after temperature shock. *bl* from 'Hunderup' and inbred HP3.	Carlsson, 1961.		L
bla	-	*blunt* leaf. Leaves have obtuse apices and reduced lobing and serration. *bla* from a mutant of 'Wis. SMR 18'.	Robinson, 1987a		W
Bt	-	*Bitter fruit*. Fruit with extreme bitter flavor. *Bt* from PI 173889 (Wild Hanzil Medicinal Cucumber).	Barham, 1953		W
bu	-	*bush*. Shortened internodes. *bu* from 'KapAhk 1'.	Pyzenkov and Kosareva, 1981		L
Bw	-	*Bacterial wilt resistance*. Resistance to *Erwinia tracheiphila*. *Bw* from PI 200818; *bw* from 'Marketer'.	Nutall and Jasmin, 1958	Robinson and Whitaker, 1974	W
by	*bu*	*bushy*. Short internodes; normal seed viability. Wild type *By* from 'Borszczagowski'; *by* from induced mutation of 'Borszczagowski'. Linked with *F* and *gy*, not with *B* or *bi*.	Kubicki et al., 1986a		?
c	-	*cream mature fruit color*. Interaction with *R* is evident in the F_2 ratio of 9 red (*RC*) : 3 orange (*Rc*) : 3 yellow (*rC*) : 1 cream (*rc*).	Hutchins, 1940		L
Cca	-	*Corynespora cassiicola resistance*. Resistance to target leaf spot; dominant to susceptibility. *Cca* from Royal Sluis Hybrid 72502; *cca* from Gy 3.	Abul-Hayja et al., 1975		W
Ccu	-	*Cladosporium cucumerinum resistance*. Resistance to scab. *Ccu* from line 127.31, a selfed progeny of 'Longfellow'; *ccu* from 'Davis Perfect'.	Bailey and Burgess, 1934	Abul-Hayja and Williams, 1976; Abul-Hayja et al., 1975, Andeweg, 1956	W

cd	-	*chlorophyll deficient*. Seedling normal at first, later becoming a light green; lethal unless grafted. *cd* from a mutant selection of backcross of MSU 713-5 x 'Midget' F1 to 'Midget'.	Burnham, et al., 1966		L
chp	-	*choripetalous*. Small first true leaf; choripetalous flowers; glossy ovary; small fruits; few seeds. Wild type *Chp* from 'Borszczagowski'; *chp* from chemically induced mutation.	Kubicki and Korzeniewska, 1984		?
cl	-	*closed flower*. Staminate and pistillate flowers do not open; male-sterile (nonfertile pollen).	Groff and Odland, 1963		W
cla	-	*Colletotrichum lagenarium resistance*. Resistance to race 1 of anthracnose; recessive to susceptibility. *Cla* from Wis. SMR 18; *cla* from SC 19B.	Abul-Hayja et al., 1978		W
Cm	-	*Corynespora melonis resistance*. Resistance to *C. melonis* dominant to susceptibility. *Cm* from 'Spotvrie'; *cm* from 'Esvier'.	van Es, 1958		?
Cmv	-	*Cucumber mosaic virus resistance*. One of several genes for resistance to CMV. *Cmv* from 'Wis. SMR 12', 'Wis. SMR 15', and 'Wis. SMR 18'; *cmv* from 'National Pickling' and 'Wis. SR 6'.	Wasuwat and Walker, 1961	Shifriss et al., 1942	W
co	-	*green corolla*. Green petals that turn white with age and enlarged reproductive organs; female-sterile. *co* from a selection of 'Extra Early Prolific'.	Hutchins, 1935	Currence, 1954	L
cor-1	-	*cordate leaves-1*. Leaves are cordate. *cor-1* from 'Nezhinskii'.	Gornitskaya, 1967		L
cor-2	*cor*	*cordate leaves-2*. Leaves are nearly round with revolute margins and no serration. Insect pollination is hindered by short calyx segments that tightly clasp the corolla, preventing full opening. *cor-2* from an induced mutant of 'Lemon'.	Robinson, 1987c		?
cp	-	*compact*. Reduced internode length, poorly developed tendrils, small flowers. *cp* from PI 308916.	Kauffman and Lower, 1976		W
cp-2	-	*compact-2*. Short internodes; small seeds; similar to *cp*, but allelism not checked. Wild type *Cp-2* from 'Borszczagowski'; *cp-2* from induced mutation of 'Borszczagowski' called W97. Not linked with *B* or *F*; interacts with *by* to produce super dwarf.	Kubicki et al., 1986b		?
cr	-	*crinkled leaf*. Leaves and seed are crinkled.	Odland and Groff, 1963a		?
cs	-	*carpel splitting*. Fruits develop deep longitudinal splits. *cs* from TAMU 1043 and TAMU 72210, which are second and fifth generation selections of MSU 3249 x SC 25.	Caruth, 1975; Pike and Caruth, 1977		?
D	*g*	*Dull fruit skin*. Dull skin of American cultivars, dominant to glossy skin of most European cultivars.	Poole, 1944; Strong, 1931; Tkachenko, 1935		W

de	*I*	*determinate habit*. Short vine with stem terminating in flowers; modified by *In-de* and other genes; degree of dominance depends on gene background. *de* from Penn 76.60G[*], Minn 158.60[*], 'Hardin's PG57'[*], 'Hardin's Tree Cucumber'[*], and S_2-1 (and inbred selection from Line 541)[**].	Denna, 1971[*]; George, 1970[**]; Hutchins, 1940	Nutall and Jasmin, 1958	W
de-2	-	*determinate-2*. Main stem growth ceases after 3 to 10 nodes, producing flowers at the apex; smooth, fragile, dark-green leaves; similar to *de*, but not checked for allelism. Wild type *De-2* from 'Borszczagowski'; *de-2* from W-sk mutant induced by ethylene-imine from 'Borszczagowski'.	Soltysiak et al., 1986		?
df	-	*delayed flowering*. Flowering delayed by long photoperiod; associated with dormancy. *df* from 'Baroda' (PI 212896)[*] and PI 215589 (*hardwickii*)[**].	Della Vecchia et al., 1982[*]; Shifriss and George, 1965[**].		W
dl	-	*delayed growth*. Reduced growth rate; shortening of hypocotyl and first internodes. *dl* from 'Dwarf Marketmore' and 'Dwarf Tablegreen', both deriving dwarfness from 'Hardin's PG-57'.	Miller and George, 1979		W
dm	*P*	*downy mildew resistance*. One of several genes for resistance to *Pseudoperonospora cubensis*. *Dm* from Sluis & Groot Line 4285; *dm* from 'Poinsett'.	van Vliet and Meysing, 1977	Jenkins, 1946; Shimizu, 1963	W
dm-1	*dm*	*downy mildew resistance-1*. One of three genes for resistance to downy mildew caused by *Pseudoperonospora cubensis* (Berk & Curt). Wild type *Dm-1* from Wisconsin SMR 18; *dm-1* from WI 4783. Not checked for allelism with *dm*.	Doruchowski and Lakowska-Ryk, 1992		?
dm-2	-	*downy mildew resistance-2*. One of three genes for resistance to downy mildew caused by *Pseudoperonospora cubensis* (Berk & Curt). Wild type *Dm-2* from Wisconsin SMR 18; *dm-2* from WI 4783. Not checked for allelism with *dm*.	Doruchowski and Lakowska-Ryk, 1992		?
dm-3	-	*downy mildew resistance-3*. One of three genes for resistance to downy mildew caused by *Pseudoperonospora cubensis* (Berk & Curt). Wild type *Dm-3* from Wisconsin SMR 18; *dm-3* from WI 4783. Not checked for allelism with *dm*.	Doruchowski and Lakowska-Ryk, 1992		?
dvl	*dl*	*divided leaf*. True leaves are partly or fully divided, often resulting in compound leaves with two to five leaflets and having incised corollas.	den Nijs and Mackiewicz, 1980		W
dvl-2	*dl-2*	*divided leaf-2*. Divided leaves after the 2nd true leaf; flower petals free; similar to *dvl*, but allelism not checked. Wild type *Dvl-2* from 'Borszczagowski'; *dvl-2* from mutant induced by ethylene-imine from 'Borszczagowski'.	Rucinska et al., 1992b		?
dw	-	*dwarf*. Short internodes. *dw* from an induced mutant of 'Lemon'.	Robinson and Mishanec, 1965		?

dwc-1	-	*dwarf cotyledons-1*. Small cotyledons; late germination; small first true leaf; died after 3rd true leaf. Wild type *Dwc-1* from 'Nishiki Suyo'; *dwc-1* from M_2 line from pollen irradiation.	Iida and Amano, 1990, 1991		?
dwc-2	-	*dwarf cotyledons-2*. Small cotyledons; late germination; small first true leaf. Wild type *Dwc-2* from 'Nishiki Suyo'; *dwc-2* from M_2 line from pollen irradiation.	Iida and Amano, 1990, 1991		?
Es-1	-	*Empty chambers-1*. Carpels of fruits separated from each other, leaving a small to large cavity in the seed cell. *Es-1* from PP-2-75; *es-1* from Gy-30-75.	Kubicki and Korzeniewska, 1983		?
Es-2	-	*Empty chambers-2*. Carpels of fruits separated from each other, leaving a small to large cavity in the seed cell. *Es-2* from PP-2-75; *es-2* from Gy-30-75.	Kubicki and Korzeniewska, 1983		?
F	*Acr, acr^F, D, st*	*Female*. High degree of pistillate sex expression; interacts with *a* and *M*; strongly modified by environment and gene background. *F* and *f* are from 'Japanese'.	Galun, 1961; Tkachenko, 1935	Kubicki, 1965, 1969a; Poole, 1944; Shifriss, 1961	W
fa	-	*fasciated*. Plants have flat stems, short internodes, and rugose leaves. *fa* was from a selection of 'White Lemon'[*].	Robinson, 1987b[*]; Shifriss, 1950		?
Fba	-	*Flower bud abortion*. Preanthesis abortion of floral buds, ranging from 10% to 100%. *fba* from MSU 0612.	Miller and Quisenberry, 1978		?
Fdp-1	-	*Fructose diphosphatase* (E.C.# 3.1.3.11). Isozyme variant found segregating in PI 192940, 169383 and 169398; 2 alleles observed.	Meglic and Staub, 1996		P
Fdp-2	-	*Fructose diphosphatase* (E.C.# 3.1.3.11). Isozyme variant found segregating in PI 137851, 164952, 113334 and 192940; 2 alleles observed.	Meglic and Staub, 1996		P
Fl	-	*Fruit length*. Expressed in an additive fashion, fruit length decreases incrementally with each copy of *fl* (H. Munger, personal communication).	Wilson, 1968		W
Foc	*Fcu-1*	*Fusarium oxysporum f. sp. cucumerinum resistance*. Resistance to fusarium wilt races 1 and 2; dominant to susceptibility. *Foc* from WIS 248; *foc* from 'Shimshon'.	Netzer et al., 1977; Vakalounakis, 1993, 1995, 1996		W
G2dh	-	*Glutamine dehydrogenase* (E.C.# 1.1.1.29). Isozyme variant found segregating in PI 285606; 5 alleles observed.	Knerr and Staub, 1992		P
g	-	*golden leaves*. Golden color of lower leaves. *G* and *g* are both from different selections of 'Nezhin'.	Tkachenko, 1935		?
gb	*n*	*gooseberry fruit*. Small, oval-shaped fruit. *gb* from the 'Klin mutant'.	Tkachenko, 1935		?
gc	-	*golden cotyledon*. Butter-colored cotyledons; seedlings die after 6 to 7 days. *gc* from a mutant of 'Burpless Hybrid'.	Whelan, 1971		W

gi	-	*ginkgo*. Leaves reduced and distorted, resembling leaves of Ginkgo; male- and female-sterile. Complicated background: It was in a segregating population whose immediate ancestors were offspring of crosses and backcrosses involving 'National Pickling', 'Chinese Long', 'Tokyo Long Green', 'Vickery', 'Early Russian', 'Ohio 31' and an unnamed white spine slicer.	John and Wilson, 1952		L
gi-2	-	*ginkgo-2*. Spatulate leaf blade with reduced lobing and altered veins; recognizable at the 2nd true leaf stage; similar to gi, fertile instead of sterile. Wild type *Gi-2* from 'Borszczagowski'; *gi-2* from mutant in the Kubicki collection.	Rucinska et al., 1992b		?
gig	-	*gigantism*. First leaf larger than normal. Wild type *Gig* from 'Borszczagowski'; *gig* from chemically induced mutation.	Kubicki et al., 1984		?
gl	-	*glabrous*. Foliage lacking trichomes; fruit without spines. Iron-deficiency symptoms (chlorosis) induced by high temperature. *gl* from NCSU 75[*] and M834-6[**].	Robinson and Mishanec, 1964[*]	Inggamer and de Ponti, 1980[**]; Robinson, 1987b	W
glb	-	*glabrate*. Stem and petioles glabrous, laminae slightly pubescent. *glb* from 'Burpless Hybrid'.	Whelan, 1973		W
gn	-	*green mature fruit*. Green mature fruits when *rr gngn*; cream colored when *rr GnGn*; orange when *R_ __*. Wild type *Gn* from 'Chipper', SMR 58 and PI 165509; *gn* from TAMU 830397.	Peterson and Pike, 1992		W
Gpi-1	-	*Glucose phosphate isomerase* (E.C.# 5.3.1.9). Isozyme variant found segregating (1 and 2) in PI 176524, 200815, 249561, 422192, 432854, 436608; 3 alleles observed.	Knerr and Staub, 1992		P
Gr-1	-	*Glutathione reductase-1* (E.C.# 1.6.4.2). Isozyme variant found segregating in PI 109275; 5 alleles observed.	Knerr and Staub, 1992		P
gy	-	*gynoecious*. Recessive gene for high degree of pistillate sex expression.	Kubicki, 1974		W
H	-	*Heavy netting of fruit*. Dominant to no netting and completely linked or pleiotropic with black spines (*B*) and red mature fruit color (*R*).	Hutchins, 1940; Tkachenko, 1935		W
hl	-	*heart leaf*. Heart shaped leaves. Wild type *Hl* from Wisconsin SMR 18; *hl* from WI 2757. Linked with *ns* and *ss* in the linkage group with *Tu-u-D-pm*.	Vakalounakis, 1992		W
hn	-	*horn like cotyledons*. Cotyledons shaped like bull horns; true leaves with round shape rather than normal lobes; circular rather than ribbed stem cross section; divided petals; spineless fruits; pollen fertile, but seed sterile. Wild type *Hn* from 'Nishiki-suyo'; *hn* from M_2 line from pollen irradiation.	Iida and Amano, 1990, 1991		?
hsl	-	*heart shaped leaves*. Leaves heart shaped rather than lobed; tendrils branched. Wild type *Hsl* from 'Nishiki-suyo'; *hsl* from M_2 line from pollen irradiation.	Iida and Amano, 1990, 1991		?

I	-	*Intensifier of P*. Modifies effect of *P* on fruit warts in *Cucumis sativus* var. *tuberculatus*.	Tkachenko, 1935		?
Idh	-	*Isocitrate dehydrogenase* (E.C.# 1.1.1.42). Isozyme variant found segregating in PI 183967, 215589; 2 alleles observed.	Knerr and Staub, 1992		P
In-de	*In(de)*	*Intensifier of* de. Reduces internode length and branching of *de* plants. *In-de* and *in-de* are from different selections (S_5-1 and S_5-6, respectively) from a determinant inbred S_2-1, which is a selection of line 541.	George, 1970		?
In-F	*F*	*Intensifier of female sex expression*. Increases degree of pistillate sex expression of *F* plants. *In-F* from monoecious line 18-1; *in-F* from MSU 713-5.	Kubicki, 1969b		?
l	-	*locule number*. Many fruit locules and pentamerous androecium; five locules recessive to the normal number of three.	Youngner, 1952		W
lg-1	-	*light green cotyledons-1*. Light green cotyledons, turning dark green; light green true leaves, turning dark green; poorly developed stamens. Wild type *Lg-1* from 'Nishiki-suyo'; *lg-1* from M_2 line from pollen irradiation.	Iida and Amano, 1990, 1991		?
lg-2	-	*light green cotyledons-2*. Light green cotyledons, turning dark green (faster than lg-1; light green true leaves, turning dark green; normal stamens. Wild type *Lg-2* from 'Nishiki-suyo'; *lg-2* from M_2 line from pollen irradiation.	Iida and Amano, 1990, 1991		?
lh	-	*long hypocotyl*. As much as a 3-fold increase in hypocotyl length. *lh* from a 'Lemon' mutant.	Robinson and Shail, 1981		W
ll	-	*little leaf*. Normal-sized fruits on plants with miniature leaves and smaller stems. *ll* from Ark. 79-75.	Goode et al., 1980; Wehner et al., 1987		W
ls	-	*light sensitive*. Pale and smaller cotyledons, lethal at high light intensity. *ls* from a mutant of 'Burpless Hybrid'.	Whelan, 1972b		L
ls	*gc*	*light sensitive*. Yellow cotyledons, lethal in high light. Abstract gave *gc* as symbol; article that followed gave *ls* as symbol. Mutant *ls* from a selection of 'Burpless Hybrid'.	Whelan, 1971, 1972		?
m	*a, g*	*andromonoecious*. Plants are andromonoecious if (*mf*); monoecious if (*Mf*); gynoecious if (*MF*) and hermaphroditic if (*mF*). *m* from 'Lemon'[*].	Rosa, 1928[*]; Tkachenko, 1935	Shifriss, 1961; Wall, 1967; Youngner, 1952	W
m-2	*h*	*andromonoecious-2*. Bisexual flowers with normal ovaries.	Iezzoni, 1982; Kubicki, 1974		?
Mdh-1	-	*Malate dehydrogenase-1* (E.C.# 1.1.1.37). Isozyme variant found segregating in PI 171613, 209064, 326594; 3 alleles observed.	Knerr and Staub, 1992		P
Mdh-2	-	*Malate dehydrogenase-2* (E.C.# 1.1.1.37). Isozyme variant found segregating in PI 174164, 185690, 357835, 419214; 2 alleles observed.	Knerr and Staub, 1992		P
Mdh-3	-	*Malate dehydrogenase-3* (E.C.# 1.1.1.37).	Knerr et al., 1995		P

Mdh-4	*Mdh-3*	*Malate dehydrogenase-4* (E.C.# 1.1.1.37). Isozyme variant found segregating in PI 255236, 267942, 432854, 432887; 2 alleles observed.	Knerr and Staub, 1992		P
mj		A single recessive gene for resistance to the root-knot nematode (*Meloidogyne javanica*) from *Cucumis sativus* var. *hardwickii*; *mj* from NC-42 (LJ 90430).	Walters et al., 1996; 1997	Walters and Wehner, 1998	W
mp	pf^{+}, pf^{d}, pf^{p}	*multi-pistillate*. Several pistillate flowers per node, recessive to single pistillate flower per node. *mp* from MSU 604G and MSU 598G.	Nandgaonkar and Baker, 1981	Fujieda et al., 1982	W
Mp-2	-	*Multi-pistillate-2*. Several pistillate flowers per node. Single dominant gene with several minor modifiers. *Mp-2* from MSU 3091-1.	Thaxton, 1974		?
Mpi-1	-	*Mannose phosphate isomerase* (E.C.# 5.3.1.8). Isozyme variant found segregating in PI 176954, and 249562; 2 alleles observed.	Meglic and Staub, 1996		P
Mpi-2	-	*Mannose phosphate isomerase* (E.C.# 5.3.1.8). Isozyme variant found segregating in PI 109275, 175692, 200815, 209064, 263049, 354952; 2 alleles observed.	Knerr and Staub, 1992		P
mpy	*mpi*	*male pygmy*. Dwarf plant with only staminate flowers. Wild type *Mpy* from Wisconsin SMR 12; *mpy* from Gnome 1, a selection of 'Rochford's Improved'.	Pyzhenkov and Kosareva, 1981		?
ms-1	-	*male sterile-1*. Staminate flowers abort before anthesis; partially female-sterile. *ms-1* from selections of 'Black Diamond' and 'A & C'.	Shifriss, 1950	Robinson and Mishanec, 1967	L
ms-2	-	*male sterile-2*. Male-sterile; pollen abortion occurs after first mitotic division of the pollen grain nucleus. *ms-2* from a mutant of 'Burpless Hybrid'.	Whelan, 1973		?
$ms\text{-}2^{(PS)}$	-	*male sterile-2 pollen sterile*. Male-sterile; allelic to *ms-2*, but not to *ap*. $ms\text{-}2^{(PS)}$ from a mutant of Sunseeds 23B-X26.	Zhang et al., 1994		?
mwm	-	Moroccan watermelon mosaic virus resistance single recessive gene from Chinese cucumber cultivar 'TMG-1'	Kabelka and Grumet, 1997		W
n	-	*negative geotropic peduncle response*. Pistillate flowers grow upright; *n* from 'Lemon'; *N* produces the pendant flower position of most cultivars.	Odland, 1963b		W
ns	-	*numerous spines*. Few spines on the fruit is dominant to many. *ns* from Wis. 2757.	Fanourakis, 1984; Fanourakis and Simon, 1987		W
O	*y*	*Orange-yellow corolla*. Orange-yellow dominant to light yellow. *O* and *o* are both from 'Nezhin'.	Tkachenko, 1935		?
opp	-	*opposite leaf arrangement*. Opposite leaf arrangement is recessive to alternate and has incomplete penetrance. *opp* from 'Lemon'.	Robinson, 1987e		W
P	-	*Prominent tubercles*. Prominent on yellow rind of *Cucumis sativus* var. *tuberculatus*, incompletely dominant to brown rind without tubercles. *P* from 'Klin'; *p* from 'Nezhin'.	Tkachenko, 1935		W

Pc	*P*	*Parthenocarpy*. Sets fruit without pollination. *Pc* from 'Spotvrie'*; *pc* from MSU 713-205*.	Pike and Peterson, 1969; Wellington and Hawthorn, 1928; Whelan, 1973	de Ponti and Garretsen, 1976	?
Pe	-	*Palisade epidermis*. Epidermal cells arranged perpendicular to the fruit surface. Wild type *Pe* from 'Wisconsin SMR 18', 'Spartan Salad' and Gy 2 compact; *pe* from WI 2757.	Fanourakis and Simon, 1987		W
Pep-gl-1	-	*Peptidase with glycyl-leucine* (E.C.# 3.4.13.11). Isozyme variant found segregating in PI 113334, 212896; 2 alleles observed.	Meglic and Staub, 1996		P
Pep-gl-2	-	*Peptidase with glycyl-leucine* (E.C.# 3.4.13.11). Isozyme variant found segregating in PI 137851, 212896; 2 alleles observed.	Meglic and Staub, 1996		P
Pep-la	-	*Peptidase with leucyl-leucine* (E.C.# 3.4.13.11). Isozyme variant found segregating in PI 169380, 175692, 263049, 289698, 354952; 5 alleles observed.	Knerr and Staub, 1992		P
Pep-pap	-	*Peptidase with phenylalanyl-L-proline* (E.C.# 3.4.13.11). Isozyme variant found segregating in PI 163213, 188749, 432861; 2 alleles observed.	Knerr and Staub, 1992		P
Per-4	-	*Peroxidase* (E.C.# 1.11.1.7). Isozyme variant found segregating in PI 215589; 2 alleles observed.	Knerr and Staub, 1992		P
Pgd-1	-	*Phosphogluconate dehydrogenase-1* (E.C.# 1.1.1.43). Isozyme variant found segregating in PI 169380, 175692, 222782; 2 alleles observed.	Knerr and Staub, 1992		P
Pgd-2	-	*Phosphogluconate dehydrogenase-2* (E.C.# 1.1.1.43). Isozyme variant found segregating in PI 171613, 177364, 188749, 263049, 285606, 289698, 354952, 419214, 432858; 2 alleles observed.	Knerr and Staub, 1992		P
Pgm-1	-	*Phosphoglucomutase* (E.C.# 5.4.2.2). Isozyme variant found segregating in PI 171613, 177364, 188749, 263049, 264229, 285606, 289698, 354952; 2 alleles observed.	Knerr and Staub, 1992		P
pl	-	*pale lethal*. Slightly smaller pale-green cotyledons; lethal after 6 to 7 days. *Pl* from 'Burpless Hybrid'; *pl* from a mutant of 'Burpless Hybrid'.	Whelan, 1973		L
pm-1	-	*powdery mildew resistance-1*. Resistance to *Sphaerotheca fuliginia*. *pm-1* from 'Natsufushinari'.	Fujieda and Akiya, 1962; Kooistra, 1971	Shanmugasundarum et al., 1972	?
pm-2	-	*powdery mildew resistance-2*. Resistance to *Sphaerotheca fuliginia*. *pm-2* from 'Natsufushinari'.	Fujieda and Akiya, 1962; Kooistra, 1971	Shanmugasundarum et al., 1972	?
pm-3	-	*powdery mildew resistance-3*. Resistance to *Sphaerotheca fuliginia*. *pm-3* found in PI 200815 and PI 200818.	Kooistra, 1971	Shanmugasundarum et al., 1972	W
pm-h	*s, pm*	*powdery mildew resistance expressed by the hypocotyl*. Resistance to powdery mildew as noted by no fungal symptoms appearing on seedling cotyledons is recessive to susceptibility. *Pm-h* from 'Wis. SMR 18'; *pm-*	Fanourakis, 1984; Shanmugasundarum et al., 1971b		W

		h from 'Gy 2 *cp cp*', 'Spartan Salad', and Wis. 2757.			
pr	-	*protruding ovary*. Exerted carpels. *pr* from 'Lemon'.	Youngner, 1952.		W
prsv	*wmv-1-1*	*watermelon mosaic virus 1 resistance*. Resistance to papaya ringspot virus (formerly watermelon mosaic virus 1). Wild type *Prsv* from WI 2757; *prsv* from 'Surinam'.	Wang et al., 1984		
Prsv-2		Resistance to papaya ringspot virus; *Prsv-2* from TMG-1.	Wai and Grumet, 1995	Wai et al., 1997	W
psl	*pl*	Pseudomonas lachrymans *resistance*. Resistance to *Pseudomonas lachrymans* is recessive. *Psl* from 'National Pickling' and 'Wis. SMR 18'; *psl* from MSU 9402 and Gy 14.	Dessert et al., 1982		W
R	-	*Red mature fruit*. Interacts with *c*; linked or pleiotropic with *B* and *H*.	Hutchins, 1940		W
rc	-	*revolute cotyledon*. Cotyledons are short, narrow, and cupped downwards; enlarged perianth. *rc* from 'Burpless Hybrid' mutant.	Whelan et al., 1975		L
rc-2		recessive gene for revolute cotyledons; rc-2 fro NCG-0093 (short petiole mutant)	Wehner et al., 1998b		W
ro	-	*rosette*. Short internodes, muskmelon-like leaves. *ro* from 'Megurk', the result of a cross involving a mix of cucumber and muskmelon pollen.	de Ruiter et al., 1980		W
s	*f, a*	*spine size and frequency*. Many small fruit spines, characteristic of European cultivars is recessive to the few large spines of most American cultivars.	Strong, 1931; Tkachenko, 1935	Caruth, 1975; Poole, 1944	W
s-2	-	*spine-2*. Acts in duplicate recessive epistatic fashion with *s-3* to produce many small spines on the fruit. *s-2* from Gy 14; *s-2* from TAMU 72210.	Caruth, 1975		?
s-3	-	*spine-3*. Acts in duplicate recessive epistatic fashion with *s-2* to produce many small spines on the fruit. *S-3* from Gy 14; *s-3* from TAMU 72210.	Caruth, 1975		?
sa	-	*salt tolerance*. Tolerance to high salt levels is attributable to a major gene in the homozygous recessive state and may be modified by several minor genes. *Sa* from PI 177362; *sa* from PI 192940.	Jones, 1984		P
sc	*cm*	*stunted cotyledons*. Small, concavely curved cotyledons; stunted plants with cupped leaves; abnormal flowers. *Sc sc* from Wis. 9594 and 9597.	Shanmugasundarum and Williams, 1971; Shanmugasundarum et al., 1972.		W
Sd	-	*Sulfur dioxide resistance*. Less than 20% leaf damage in growth chamber. *Sd* from 'National Pickling'; *sd* from 'Chipper'.	Bressan et al., 1981		W
sh	-	*short hypocotyl*. Hypocotyl of seedlings 2/3 the length of normal. Wild type *Sh* from 'Borszczagowski'; *sh* from khp, an induced mutant from 'Borszczagowski'.	Soltysiak and Kubicki 1988		?

shl	-	*shrunken leaves*. First and 2nd true leaves smaller than normal; later leaves becoming normal; slow growth; often dying before fruit set. Wild type *Shl* from 'Nishiki-suyo'; *shl* from M_2 line from pollen irradiation.	Iida and Amano, 1990, 1991		?
Skdh	-	*Shikimate dehydrogenase* (E.C.# 1.1.1.25). Isozyme variant found segregating in PI 302443, 390952, 487424; 2 alleles observed.	Meglic and Staub, 1996		P
sp	-	*short petiole*. Leaf petioles of first nodes 20% the length of normal. *sp* from Russian mutant line 1753.	den Nijs and de Ponti, 1983		W
sp-2	-	*short petiole-2*. Leaf petioles shorter, darker green than normal at 2-leaf stage; crinkled leaves with slow development; short hypocotyl and stem; little branching. Not tested for allelism with *sp*. Wild type *Sp-2* from 'Borszczagowski'; *sp-2* from chemically induced mutation.	Rucinska et al., 1992a		?
ss	-	*small spines*. Large, coarse fruit spines is dominant to small, fine fruit spines. *Ss* from 'Spartan Salad', 'Wis. SMR 18' and 'GY 2 *cp cp*'; *ss* from Wis. 2757.	Fanourakis, 1984; Fanourakis and Simon, 1987		W
T	-	*Tall plant*. Tall incompletely dominant to short.	Hutchins, 1940		?
td	-	*tendrilless*. Tendrils lacking; associated with misshapen ovaries and brittle leaves. *Td* from 'Southern Pickler'; *td* from a mutant of 'Southern Pickler'.	Rowe and Bowers, 1965		W
te	-	*tender skin of fruit*. Thin, tender skin of some European cultivars; recessive to thick tough skin of most American cultivars.	Poole, 1944; Strong, 1931		W
Tr	-	*Trimonoecious*. Producing staminate, perfect, and pistillate flowers in this sequence during plant development. *Tr* from Tr-12, a selection of a Japanese cultivar belonging to the Fushinari group; *tr* from H-7-25. MOA-309, MOA-303, and AH-311-3.	Kubicki, 1969d		P
Tu	-	*Tuberculate fruit*. Warty fruit characteristic of American cultivars is dominant to smooth, non-warty fruits characteristic of European cultivars.	Strong, 1931; Wellington, 1913	Andeweg, 1956; Poole, 1944	W
u	*M*	*uniform immature fruit color*. Uniform color of European cultivars recessive to mottled or stippled color of most American cultivars.	Strong, 1931	Andeweg, 1956	W
ul	-	*umbrella leaf*. Leaf margins turn down at low relative humidity making leaves look cupped. *ul* source unknown.	den Nijs and de Ponti, 1983		W
v	-	*virescent*. Yellow leaves becoming green.	Strong, 1931; Tkachenko, 1935		L
vvi	-	*variegated virescent*. Yellow cotyledons, becoming green; variegated leaves.	Abul-Hayja and Williams, 1976		L
w	-	*white immature fruit color*. White is recessive to green. *W* from 'Vaughan', 'Clark's Special', 'Florida Pickle' and 'National Pickling'; *w* from 'Bangalore'.	Cochran, 1938		W

wf	-	*White flesh*. Intense white flesh color is recessive to dingy white; acts with *yf* to produce F_2 of 12 white (*Wf Yf* and *wf Yf*) : 3 yellow (*Wf yf*) : 1 orange (*wf yf*). *Wf* from EG and G6, each being dingy white (*Wf Yf*): *wf* from 'NPI ' which is orange (*wf yf*).	Kooistra, 1971		?
wi	-	*wilty leaves*. Leaves wilting in the field, but not in shaded greenhouse; weak growth; no fruiting. Wild type *Wi* from 'Nishiki-suyo'; *wi* from M_2 line from pollen irradiation.	Iida and Amano, 1990, 1991		?
Wmv	-	*Watermelon mosaic virus resistance*. Resistance to strain 2 of watermelon mosaic virus. *Wmv* from 'Kyoto 3 Feet'; *wmv* from 'Beit Alpha'.	Cohen et al., 1971		P
wmv-1-1	-	*watermelon mosaic virus-1 resistance*. Resistance to strain 1 of watermelon mosaic virus by limited systemic translocation; lower leaves may show severe symptoms. *Wmv-1-1* from Wis. 2757; *wmv-1-1* from 'Surinam'.	Wang et al., 1984	Provvidenti, 1985	?
wmv-2	-	*watermelon mosaic virus resistance*. Expressed in the cotyledon and throughout the plant; *wmv-2* from TMG-1.	Wai et al., 1997		W
wmv-3	-	*watermelon mosaic virus resistance*. Expressed only in true leaves; *wmv-3* from TMG-1.	Wai et al., 1997		W
wmv-4	-	*watermelon mosaic virus resistance*. Expressed only in true leaves; *wmv-4* from TMG-1.	Wai et al., 1997		W
wy	-	*wavy rimed cotyledons*. Wavy rimed cotyledons, with white centers; true leaves normal. Wild type *Wy* from 'Nishiki-suyo'; *wy* from M_2 line from pollen irradiation.	Iida and Amano, 1990, 1991		?
yc-1	-	*yellow cotyledons-1*. Cotyledons yellow at first, later turning green. *yc-1* from a mutant of Ohio MR 25.	Aalders, 1959		W
yc-2	-	*yellow cotyledons-2*. Virescent cotyledons. *yc-2* from a mutant of 'Burpless Hybrid'.	Whelan and Chubey, 1973; Whelan et al., 1975		W
yf	*v*	*yellow flesh*. Interacts with *wf* to produce F_2 of 12 white (*Wf Yf* and *wf Yf*) : 3 yellow (*Wf yf*) : 1 orange (*wf yf*). *Yf* from 'Natsufushinari', which has an intense white flesh (*Yf wf*); *yf* from PI 200815 which has a yellow flesh (*yf Wf*).	Kooistra, 1971		P
yg	*gr*	*yellow-green immature fruit color*. Recessive to dark green and epistatic to light green. *yg* from 'Lemon'.	Youngner, 1952		W
yp	-	*yellow plant*. Light yellow-green foliage; slow growth.	Abul-Hayja and Williams, 1976		?
ys	-	*yellow stem*. Yellow cotyledons, becoming cream-colored; cream-colored stem, petiole and leaf veins; short petiole; short internode. Wild type *Ys* from 'Borszczagowski'; *ys* from chemically induced mutation.	Rucinska et al., 1991		?
zym-Dina	-	zucchini yellow mosaic virus resistance; *zym-Dina* from Dina-1.	Kabelka et al., 1997	Wai et al., 1997	P

zym-TMG1	*zymv*	*zucchini yellow mosaic virus resistance*. Inheritance is incomplete, but usually inherited in a recessive fashion; source of resistance is 'TMG-1'.	Provvidenti, 1987; Kabelka et al., 1997	Wai et al., 1997	W

[z] Asterisks on cultigens and associated references indicate the source of information for each.

[y] W = Mutant available through T.C. Wehner, cucumber gene curator for the Cucurbit Genetics Cooperative; P = mutants are available as standard cultivars or accessions from the Plant Introduction Collection; ? = availability not known; L = mutant has been lost.

* Isozyme nomenclature follows a modified form of Staub et al. (1985) previously described by Richmond (1972) and Gottlieb (1977).

Table 2. The cloned genes of cucumber and their function.[z]

Gene accession	Tissue source	Function	Clone type	Reference
Genes involved in seed germination or seedling development				
X85013	Cotyledon cDNA library	Encoding a T-complex protein	cDNA	Ahnert et al., 1996
AJ13371	Cotyledon cDNA library	Encoding a matrix metalloproteinases	cDNA	Delorme et al., 2000
X15425	Cotyledon cDNA library	Glyoxysomal enzyme malate synthase	Genomic DNA fragment	Graham et al., 1989; 1990
X92890	Cotyledon cDNA library	Encoding a lipid body lipoxygenase	cDNA	Höhne et al., 1996
L31899	Senescing cucumber cotyledon cDNA library	Encoding an ATP-dependent phosphoenolpyruvate carboxykinase (an enzyme of the gluconeogenic pathway)	cDNA	Kim and Smith, 1994a
L31900	Cotyledon cDNA library	Encoding microbody NAD(+)-dependent malate dehydrogenase (MDH)	cDNA	Kim and Smith, 1994b
L44134	Senescing cucumber cDNA library	Encoding a putative SPF1-type DNA binding protein	cDNA	Kim et al., 1997
U25058	Cotyledons	Encoding a lipoxygenase-1 enzyme	cDNA	Matsui et al., 1995; 1999
Y12793	Cotyledon cDNA library	Encoding a patatin like protein	cDNA	May et al., 1998
X67696	Cotyledon cDNA library	Encoding the 48539 Da precursor of thiolase	cDNA	Preisig-Muller and Kindl, 1993a
X67695	Cotyledon cDNA library	Encoding homologous to the bacterial dnaJ protein	cDNA	Preisig-Muller and Kindl, 1993b
X79365	Seedling cDNA library	Encoding glyoxysomal tetrafunctional protein	cDNA	Preisig-Muller et al., 1994
X79366	Seedling cDNA library	Encoding glyoxysomal tetrafunctional protein	cDNA	Preisig-Muller et al., 1994
Z35499	Genomic library	Encoding the glyoxylate cycle enzyme isocitrate lyase	Genomic gene	Reynolds and Smith, 1995
M59858	Cotyledon cDNA library	Encoding a stearoyl-acyl-carrier-protein (ACP) desaturase	cDNA	Shanklin and Somerville, 1991
M16219	Cotyledon cDNA library	Encoding glyoxysomal malate synthase	cDNA	Smith and Leaver, 1986
Genes involved in photosynthesis and photorespiration activities				

M16056	Cotyledon cDNA library	Encoding ribulose bisphosphate carboxylase/oxygenase	cDNA	Greenland et al., 1987
M16057	Cotyledon cDNA library	Encoding chlorophyll a/b-binding protein	cDNA	Greenland et al., 1987
M16058	Cotyledon cDNA library	Encoding chlorophyll a/b-binding protein	cDNA	Greenland et al., 1987
X14609	cotyledon cDNA library	Encoding a NADH-dependent hydroxypyruvate reductase (HPR	cDNA	Greenler et al., 1989
Y09444	Chloroplast genomic library	tRNA gene	Chloroplast DNA fragment	Hande and Jayabaskaran, 1997
X75799	Chloroplast genomic library	Chloroplast tRNA (Leu) (cAA) gene	Genomic DNA fragment	Hande et al., 1996
D50456	Cotyledon cDNA library	Encoding 17.5-kDa polypeptide of cucumber photosystem I	cDNA	Iwasaki et al., 1995
S69988	Hypocotyls	Cytoplasmic tRNA (Phe)	cytoplasmic DNA fragment	Jayabaskaran and Puttaraju, 1993
S78381	Cotyledon cDNA library	Encoding NADPH-protochlorophyllide oxidoreductase	cDNA	Kuroda et al., 1995
D26106	Cotyledon cDNA library	Encoding ferrochelatase	cDNA	Miyamoto et al., 1994
U65511	Green peelings cDNA library	Encoding the 182 amino acid long precursor stellacyanin	cDNA	Nersissian et al., 1996
AF099501	Petal cDNA library	Encoding the carotenoid-associated protein	cDNA	Ovadis et al., 1998
X67674	Cotyledon cDNA library	Encoding ribulosebisphosphate carboxylase/oxygenase activase	cDNA	Preisig-Muller and Kindl, 1992
X58542	Cucumber genomic library	Encoding NADH-dependent hydroxypyruvate reductase	Genomic DNA fragment	Schwartz et al., 1991
U62622	Seedling cDNA library	Encoding monogalacto-syldiacylglycerol synthase	cDNA	Shimojima et al., 1997
D50407	Cotyledon cDNA library	Encoding glutamyl-tRNA reductase proteins	cDNA	Tanaka et al., 1996
D67088	Cotyledon cDNA library	Encoding glutamyl-tRNA reductase proteins	cDNA	Tanaka et al., 1996
D83007	Cotyledon cDNA library	Encoding a subunit XI (psi-L) of photosystem I	cDNA	Toyama et al., 1996
Genes expressed mainly in roots.				
AB025717	Root RNA	Lectin-like xylem sap protein	cDNA	Masuda et al., 1999
U36339	Root cDNA library	Encoding root lipoxygenase	cDNA	Matsui et al., 1998
AB015173	Root cDNA library	Encoding glycine-rich protein-1	cDNA	Sakuta et al., 1998
AB015174	Root cDNA library	Encoding glycine-rich protein-1	cDNA	Sakuta et al., 1998
Flower genes				
AF035438	Female flower cDNA library	MADS box protein CUM1	cDNA	Kater et al., 1998
AF035439	Female flower cDNA library	MADS box protein CUM10	cDNA	Kater et al., 1998
D89732	Seedlings	Encoding 1-aminocyclo-propane-	cDNA	Kamachi et al.,

		1-carboxylate synthase		1997
AB003683	Seedlings	Encoding 1-aminocyclo-propane-1-carboxylate synthase	cDNA	Kamachi et al., 1997
AB003684	Seedlings	Encoding 1-aminocyclo-propane-1-carboxylate synthase	cDNA	Kamachi et al., 1997
AB035890	Fruit RNA	Encoding polygalacturonase	cDNA	Kubo et al., 2000
AF022377	Floral buds	Encoding agamous-like putative transcription factor (CAG1) mRNA	cDNA	Perl-Treves et al., 1998
AF022378	Floral buds	Encoding agamous like putative transcription factor (CAG2) mRNA	cDNA	Perl-Treves et al., 1998
AF022379	Floral buds	Encoding agamous-like putative transcription factor (CAG3) mRNA	cDNA	Perl-Treves et al., 1998
U59813	Genomic DNA	Encoding 1-aminocyclo-propane-1-carboxylate synthase	Genomic DNA fragment	Trebitsh et al., 1997
X95593	Corolla cDNA library	Encoding carotenoid-associated protein	cDNA	Vishnevetsky et al., 1996
AB026498	Shoot apex RNA	Ethylene-receptor-related gene	cDNA	Yamasaki et al., 2000
Genes involved in fruit development and maturation				
AB010922	Fruit cDNA library	Encoding the ACC synthase	cDNA	Mathooko et al., 1999
J04494	Fruit cDNA library	Encoding an ascorbate oxidase	cDNA	Ohkawa et al., 1989; 1990
AB006803	Fruit cDNA library	Encoding ACC synthase	cDNA	Shiomi et al., 1998
AB006804	Fruit cDNA library	Encoding ACC synthase	cDNA	Shiomi et al., 1998
AB006805	Fruit cDNA library	Encoding ACC synthase	cDNA	Shiomi et al., 1998
AB006806	Fruit cDNA library	Encoding ACC oxidase	cDNA	Shiomi et al., 1998
AB006807	Fruit cDNA library	Encoding ACC oxidase	cDNA	Shiomi et al., 1998
AB008846	Pollinated fruit cDNA library	Corresponding genes preferentially expressed in the pollinated fruit	cDNA	Suyama et al., 1999
AB008847	Pollinated fruit cDNA library	Corresponding genes preferentially expressed in the pollinated fruit	cDNA	Suyama et al., 1999
AB008848	Pollinated fruit cDNA library	Corresponding genes preferentially expressed in the pollinated fruit	cDNA	Suyama et al., 1999
Genes involved in cell wall loosening and cell enlargement				
AB001586	Hypocotyl RNA	Encoding homologous to serine/threonine protein kinases (for CsPK1.1)	cDNA	Chono et al., 1999
AB001587	Hypocotyl RNA	Encoding homologous to serine/threonine protein kinases (for CsPK1.2)	cDNA	Chono et al., 1999
AB001588	Hypocotyl RNA	Encoding homologous to serine/threonine protein kinases (for CsPK2.1)	cDNA	Chono et al., 1999
AB001589	Hypocotyl RNA	Encoding homologous to serine/threonine protein kinases	cDNA	Chono et al., 1999

		(for CsPK2.2)		
AB001590	Hypocotyl RNA	Encoding homologous to serine/threonine protein kinases (for CsPK3)	cDNA	Chono et al., 1999
AB001591	Hypocotyl RNA	Encoding homologous to serine/threonine protein kinases (for CsPK4.1)	cDNA	Chono et al., 1999
AB001592	Hypocotyl RNA	Encoding homologous to serine/threonine protein kinases (for CsPK4.2)	cDNA	Chono et al., 1999
AB001593	Hypocotyl RNA	Encoding homologous to serine/threonine protein kinases (for CsPK5)	cDNA	Chono et al., 1999
U30382	Hypocotyl cDNA library	Encoding expansins	cDNA	Shcherban et al., 1995
U30460	Hypocotyl cDNA library	Encoding expansins	cDNA	Shcherban et al., 1995
Genes induced or repressed by plant hormones				
D49413	Hypocotyl cDNA library	Corresponding to a gibberellin-responsive gene encoding an extremely hydrophobic protein	cDNA	Chono et al., 1996
AB026821	Seedling RNA	Encoding IAA induced nuclear proteins	cDNA	Fujii et al., 2000
AB026822	Seedling RNA	Encoding IAA induced nuclear proteins	cDNA	Fujii et al., 2000
AB026823	Seedling RNA	Encoding IAA induced nuclear proteins	cDNA	Fujii et al., 2000
M32742	Cotyledon cDNA library	Encoding ethylene-induced putative peroxidases	cDNA	Morgens et al., 1990
D29684	Cotyledon cDNA library	Cytokinin-repressed gene	cDNA	Teramoto et al., 1994
D79217	Genomic library	Cytokinin-repressed gene	Genomic DNA fragment	Teramoto et al., 1996
D63451	Cotyledon cDNA library	Homologous to Arabidopsis cDNA clone 3003	cDNA	Toyama et al., 1995
D63384	Cotyledon cDNA library	Encoding catalase	cDNA	Toyama et al., 1995
D63385	Cotyledon cDNA library	Encoding catalase	cDNA	Toyama et al., 1995
D63386	Cotyledon cDNA library	Encoding catalase	cDNA	Toyama et al., 1995
D63387	Cotyledon cDNA library	Encoding lectin	cDNA	Toyama et al., 1995
D63388	Cotyledon cDNA library	Encoding 3-hydroxy-3-methylglutaryl CoA reductase	cDNA	Toyama et al., 1995
D63389	Cotyledon cDNA library	Encoding 3-hydroxy-3-methylglutaryl CoA reductase	cDNA	Toyama et al., 1995
D63388	Cotyledon cDNA library	Encoding a basic region/helix-loop-helix protein	cDNA	Toyama et al., 1999
Resistance genes				
M84214	Genomic library	Encoding the acidic class III chitinase	cDNA	Lawton et al., 1994
M24365	Leave cDNA library	Encoding a chitinase	cDNA	Metraux et al., 1989

D26392	Seedling cDNA library	Encoding FAD-Enzyme monodehydroascorbate (MDA) reductase	cDNA	Sano and Asada, 1994
Somatic embryo gene.				
X97801	Embryogenic callus cDNA library	MADS-box gene	cDNA	Filipecki et al., 1997
Repeated DNA sequences				
X03768	Genomic DNA	Satellite type I	Genomic DNA fragment	Ganal et al., 1986
X03769	Genomic DNA	Satellite type II	Genomic DNA fragment	Ganal et al., 1986
X03770	Genomic DNA	Satellite type III	Genomic DNA fragment	Ganal et al., 1986
X69163	Genomic DNA	Satellite type IV	Genomic DNA fragment	Ganal et al., 1988a
X07991	rDNA	Ribosomal DNA intergenic spacer	Genomic DNA fragment	Ganal et al., 1988b
X51542	Cotyledons	Ribosomal DNA intergenic spacer	Genomic DNA fragment	Zentgraf et al., 1990

[z] Only the sequences published in both journals and the genebank database are listed.

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Gene Nomenclature for the Cucurbitaceae

1. Names of genes should describe a characteristic feature of the mutant type in a minimum of adjectives and/or nouns in English or Latin.
2. Genes are symbolized by italicized Roman letters, the first letter of the symbol being the same as that for the name. A minimum number of additional letters are added to distinguish each symbol.
3. The first letter of the symbol and name is capitalized if the mutant gene is dominant,. All letters of the symbol and name are in lower case if the mutant gene is recessive, with the first letter of the symbol capitalized for the dominant or normal allele. (Note: For CGC *research articles*, the normal allele of a mutant gene may be represented by the symbol "+", or the symbol of the mutant gene followed by the superscript "+", if greater clarity is achieved for the manuscript.)
4. A gene symbol shall not be assigned to a character unless supported by statistically valid segregation data for the gene.
5. Mimics, i.e. different mutants having similar phenotypes, may either have distinctive names and symbols or be assigned the same gene symbol, followed by a hyphen and distinguishing Arabic numeral or Roman letter printed at the same level as the symbol. The suffix "-1" is used, or may be understood and not used, for the original gene in a mimic series. It is recommended that allelism tests be made with a mimic before a new gene symbol is assigned to it.
6. Multiple alleles have the same symbol, followed by a Roman letter or Arabic number superscript. Similarities in phenotype are insufficient to establish multiple alleles; the allelism test must be made.
7. Indistinguishable alleles, i.e. alleles at the same locus with identical phenotypes, preferably should be given the same symbol. If distinctive symbols are assigned to alleles that are apparent re-occurrences of the same mutation, however, they shall have the same symbol with distinguishing numbers or letters in parentheses as superscripts.
8. Modifying genes may have a symbol for an appropriate name, such as intensifier, suppressor, or inhibitor, followed by a hyphen and the symbol of the allele affected. Alternatively, they may be given a distinctive name unaccompanied by the symbol of the gene modified.
9. In cases of the same symbol being assigned to different genes, or more than one symbol designated for the same gene, priority in publication will be the primary criterion for establishing the preferred symbol. Incorrectly assigned symbols will be enclosed in parentheses on the gene lists.
10. The same symbol shall not be used for nonallelic genes of different *Cucurbita* species. Allelic genes of compatible species are designated with the same symbol for the locus.

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Ignart, Frederic Centre de Recherche TEZIER, Route de Beaumont, Domaine de Maninet, 26000 Valence, France. Ph: (33) 75575757; Fax: (33) 75552681; Email: *frederic.ignart@tezier.com*. Squash and melon breeding.

Ikegami, Takayuki Sakata Seed Corp., 1743-2 Yoshioka, Kakegawa, Shizuoka, 436-0115, Japan. Ph: 81-0537-26-1111; Fax: 81-0537-26-1110. Cell biology.

Ito, Kimio Vegetable Breeding Laboratory, Hokkaido Natl. Agric. Expt. Sta., Hitsujigaoka, Sapporo, Japan 062-8555. Ph: 011(851)9141; Fax: 011(859)2178; Email: *kito@cryo.affrc.go.jp*.

Jahn, Molly Kyle Cornell Univ, Dept Plant Brdng, 312 Bradfield Hall, Ithaca, NY, 14853-1902. Ph: (607) 255-8147; Fax: (607) 255-6683; Email: *mmk9@cornell.edu*. Melon and squash breeding and genetics.

Jain, Jaagrati B-149 M. P. Enclave, Pitampura, Delhi-110034, India. Email: *jaagratijain@rediffmail.com*. Melon genetics & tissue culture.

Johnston, Rob, Jr. Johnny's Selected Seeds, Foss Hill Road, Albion, ME, 04910-9731. Ph: (207) 437-9294; Fax: (207) 437-2603; Email: *rob@johnnyseeds.com*. Squash and pumpkins.

Kampmann, Hans Henrik Breeding Station Danefeld, Odensevej 82, 5290 Marslev, Denmark. Ph: 65 95 17 00; Fax: 65 95 12 93.

Kanda, Minoru Kanda Seed Co., Ltd., 262 Shinga, Kashihara, Nara, 634-0006, Japan. Ph: 0744-22-2603; Fax: 0744-22-9073; Email: *minojazz@mue.biglobe.ne.jp*.

Karchi, Zvi 74 Hashkedim St., Kiryat-Tivon 36501, Israel. Ph: 04-9830107; Fax: 972-4-9836936. Cucurbit breeding, cucurbit physiology.

Kato, Kenji Fac. Agriculture, Okayama Univ., 1-1-1 Tsushima Naka, Okayama, 700, Japan. Ph: 81-86-251-8323; Fax: 81-86-254-0714; Email: *kenkato@cc.okayama-u.ac.jp*. Use of molecular markers for QTL mapping and cultivar identification in melon.

Katzir, Nurit Newe Ya'ar Research Center, ARO, P.O. Box 1021, Ramat Yishay, 30095, Israel. Ph: 972-4-9539554; Fax: 972-4-9836936; Email: *nuritkat@netvision.net.il*.

Keita, Sugiyama Kurume Branch, Natl Res Inst, Veg/OrnPlnts/Tea, Kurume, Fukuoka 839-8503, Japan. Ph: +81-942-43-8271; Fax: +81-942-43-7014. Watermelon.

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King, Joseph J. Seminis Vegetable Seeds, Inc., 37437 State Highway 16, Woodland, CA, 95695. Ph: (530) 666-6262; Fax: (530) 666-5759; Email: *joe.king@svseeds.com*. Genetics and breeding of melon, cucumber and squash.

King, Stephen R. Seminis Vegetable Seeds, Inc., 37437 State Hwy. 16, Woodland, CA, 95695. Ph: (530) 666-0931; Fax: (530) 668-0219; Email: *stephen.king@seminis.com*. Melon breeding.

Kirkbride, Joseph H., Jr. USDA-ARS, Systematic Bot & Mycol Lab, Rm 304, Bldg 011A, BARC-West, Beltsville, MD, 20705-2350. Ph: (301) 504-9447; Fax: (301) 504-5810; Email: *jkirkbri@asrr.arsusda.gov*. Systematic taxonomy of the Cucurbitaceae.

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Kraakman, Peter DeRuiter Zohen, Torre Caribe 7D, Aguadulce (Almeria), Spain. Email: *Peter.Kraakman@deruiterseeds.com*.

Křístková, Eva Res Inst Crop Prod, Praha-Ruzyne, Workplace Olomouc, Slechtitelu 11, 738 71 Olomouc, Czech Republic. Ph: +420-68-5228355; Fax: +420-68-5228355; Email: *olgeba@ova.pvtnet.cz*. Gene bank curating of cucurbitaceous vegetables; powdery mildew resistance in *Cucurbita*.

Kuginuki, Yasuhisa National Institute Veg/Orn/Tea, Crop Research Station, Ano, Mie 514-2392, Japan. Ph: 0592-68-1331; Fax: 0592-68-1339. Breeding for resistance to disease.

Kuhlmann, Hubert Fink GmbH, Benzstrasse 25, D-71083 Herrenberg, Germany. Ph: (07032) 922-122; Fax: (07032) 922-202.

Kuti, Joseph O. Dept Agron & Res Sci, Hort Crops Lab, Texas A&M University - Kingsville, Kingsville, TX, 78363. Ph: (361) 593-3978; Fax: (361) 593-3788; Email: *j-kuti@tamuk.edu*. Breeding and genetics; host-parasite interrelationships; postharvest physiology.

Kwack, Soo Nyeon Dept Hort Breeding, Mokpo Natl Univ, Dorimri, Chonggyemyun, Muangun, Chonnam 534-729, Korea.

Kwon, Young-Seok Natl. Alpine Agric. Expt. Sta., 1 Hoengkeri, Doam, Pyongchang, Kangwondo, Rep. Korea 232-950. Ph: 82-374-330-7811; Fax: 82-374-330-7715; Email: *yskwon@naaes.go.kr*. Watermelon germplasm evaluation and breeding for disease resistance.

Lebeda, Aleš Palacky University, Dept. Botany, Slechtitelu 11, 783 71 Olumouc, Czech Republic. Ph: +420/68/5228825; Fax: +420/68/5241027; Email: *lebeda@risc.upol.cz*.

Lee, Do-Hyon Novartis Seeds Co., Ltd., 8th fl. SungAm Bldg. #114, Nonhyun- dong, Kangnam-ku, Seoul, Korea 135-010. Ph: +82 2 3218 5400; Fax: +82 2 516 2286. Disease resistance.

Lee, Sang Yeb Breeding Res. Inst., Dongbuhannong Chem., #481-3, Deng Bong-RT, YangSeong-Myun, An Seong, Kyung Ki, South Korea 456-930. Ph: 31-674-6911-5; Fax: 31-674-6916; Email: *syleehan@hanmail.net*.

Legg, Erik 7240 Holsclaw Road, Gilroy, CA, 95020. Ph: 408-846-2228; Fax: 408- 848-8129; Email: *erik.legg@syngenta.com*. Genetics; phylogeny, resistance, molecular markers.

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Lelley, Tamas Inst Agrobiotech, Dept Plant Biotech, Konrad Lorenz Str. 20, Tulln, Lower

Austria, Austria 3430. Ph: +43 2272 66280 204; Fax: +43 2272 66280 77; Email: *lelley@ifa-tull.ac.at*. *Cucurbita* spp.

Lester, Gene USDA/ARS, Subtropical Agric Res Lab, 2301 S. International Blvd., Weslaco, TX, 78596. Ph: (210) 565-2647; Fax: (210) 565-6133; Email: *glester@pop.tamu.edu*. Stress and pre/postharvest physiology of melons.

Levi, Amnon U.S. Vegetable Laboratory, 2875 Savannah Highway, Charleston, SC, 29414. Ph: (843) 556-0840; Fax: (834) 763-7013; Email: *alevi@awod.com*.

Lin, Depei Sichuan Academic Agric. Science, Institute of Horticulture, Chengdu 610066, People's Rep. China. Ph: (028) 4791732; Fax: (028) 4442025. Watermelon, melon and *Cucurbita* breeding.

Liu, Wenge Zhengzhou Fruit Research Inst, Chinese Academy of Agric Sci, Zhengzhou, Henan, P.R. China 450009. Ph: (0371) 6815703; Fax: (0371) 6815771; Email: *wlirong@public2.zz.ha.cn*. Watermelon breeding, male sterility, tetraploids, triploids.

Lopez Anido, Fernando Catedra de Genetica, Fac. de Cs. Agrarias, UNR, CC 14, 2123 Zavalla, Argentina. Ph: 54-41-970080; Fax: 54-41-970085; Email: *felopez@fcagr.unr.edu.ar*. Breeding of *Cucurbita pepo* L. (caserta type).

Love, Stephen Loyd Aberdeen R&E Center, P.O. Box AA, Aberdeen, ID, 83210. Ph: (208) 397-4181; Fax: (208) 397-4311; Email: *slove@uidaho.edu*. Small scale private watermelon breeding with emphasis on adaptation to cold climates.

Lower, Richard L. Coll. Agriculture, Univ. Wisconsin, 1450 Linden Drive, Room 240, Madison, WI, 53706. Ph: (608) 262-2349; Fax: (608) 265-6434; Email: *richard.lower@ccmail.adp.wisc.edu*. Effects of plant type genes on yield, sex- expression, growth parameters, pest resistance & adaptability.

Loy, J. Brent Dept. Plant Biology, Univ. New Hampshire, Durham, NH, 03824. Ph: (603) 862-3216; Fax: (603) 862-4757; Email: *jbloy@cisunix.unh.edu*. Squash, melon, pumpkin. Genetics, breeding, plasticulture, mulch, rowcovers.

Maluf, Wilson Roberto Dept. de Agricultura/UFLA, Caixa Postal 37, 37200-000 Lavras-MG, Brazil. Ph: (035) 829-1326; Fax: (035) 829-1301; Email: *wrmaluf@ufla.br*. Cucumbers, melons, squashes.

Markiewicz-Ladd, Krystyna Polonica International, P.O. Box 2305, Gilroy, CA, 95021. Ph: (408) 842-1022; Fax: (408) 842-1022; Email: *polonica@aol.com*. Melons - breeding, new germplasm, postharvest physiology, biotechnology, cultural practices, new diseases.

Martyn, Ray D. Dept. Botany & Plant Pathology, 1155 Lilly Hall, Purdue Univ., West Lafayette, IN, 47907-1155. Ph: (765) 494-4615; Fax: (765) 494-0363; Email: *Martyn@btny.purdue.edu*. Soilborne diseases of watermelon and melon, particularly the Fusarium wilts and vine declines.

Matsuura, Seiji Kiyohara Breeding Sta., Tohoku Seed Co., 1625 Nishihara, Himuro, Utsunomiya, Japan. Ph: 0286-34-5428; Fax: 0286-35-6544.

Mayberry, Mella-Dee Seminis Vegetable Seeds, 37437 State Hwy 16, Woodland, CA, 95695. Ph: (530) 669-6185; Fax: (530) 666-2308; Email: *Mella-Dee.Mayberry@seminis.com*.

Maynard, Donald N. University of Florida, 5007 60th Street East, Bradenton, FL, 34203. Ph: (941) 751-7636; Fax: (941) 751-7639; Email: *bra@gnv.ifas.ufl.edu*. Tropical *C. moschata* improvement; watermelon variety evaluation and production practices.

McClurg, Charles A. University of Maryland, Dept. Natural Resource Sci., College Park, MD, 20742-4452. Ph: (301) 405-4342; Fax: (301) 314-9308; Email: *cm19@umail.umd.edu*. Production and culture of cucurbit crops.

McCreight, J.D. USDA-ARS, 1636 E. Alisal St., Salinas, CA, 93905. Ph: (831) 755-2864; Fax: (831) 755-2814; Email: *VegBreeder@netscape.net*. Melon breeding and genetics.

McGrath, Desmond John Dept. Primary Ind., Hortic. Res. Sta., P.O. Box 538, Bowen, Queensland 4805, Australia. Ph: +61-7-4785 2255; Fax: +61-7-4785 2427; Email: *mcgratdj@prose.dpi.qld.gov.au*. Disease resistance in *Cucumis melo*, particularly gummy stem blight.

Meadows, Mike Syngenta Seeds, Inc., 10290 Greenway Road, Naples, FL, 34114. Ph: (941) 775-4090; Fax: (941) 774-6852; Email: *mike.meadows@syngenta.com*. Vegetable diseases.

Merrick, Laura C. Dept. Agron., Iowa St. Univ, G207 Agronomy Hall, Ames, IA, 50011-1010. Ph: (515) 294-7636; Fax: (515) 294-3163; Email: *lmerrick@iastate.edu*. *Cucurbita* evolution; cucurbit

germplasm evaluation and conservation; ethnobotany and evolution.

Mochizuki, Tatsuya Kurume Br, Natl Res Inst Veg/Orn/Tea, 1823 Mii-machi, Kurume, Fukuoka 830, Japan. Ph: 0942-43-8271; Fax: 0942-43-7014.

Morelock, Ted Dept. Horticulture & Forestry, University of Arkansas, Fayetteville, AR, 72701. Ph: (501) 575-2603; Fax: (501) 575-8619; Email: *morelock@comp.uark.edu*. Cucumber breeding.

Munger, H.M. Cornell University, 252 Emerson Hall, Ithaca, NY, 14853. Ph: (607) 255-7820; Fax: (607) 255-6683; Email: *hmm11@cornell.edu*. Cucurbit breeding and disease resistance.

Myers, James R. Dept. Horticulture, Oregon State Univ., 4137 Ag Life Sciences Bldg., Corvallis, Oregon, 97331. Ph: (541) 737-3083; Email: *myersja@bcc.orst.edu*.

Nadel, Michael 10851 Woodbine Street, Los Angeles, CA, 90034. Ph: (310) 838- 7675; Fax: (310) 202-7466; Email: *dansonseed@mediaone.net*. Breeding summer squash, cucumbers, melons and watermelons.

Nannes, Jeroen Seminis Vegetable Seeds, P.O. Box 93, 2675 ZH Honselersdijk, The Netherlands. Email: *jnannes@svseeds.nl*. Breeding slicing cucumber.

Navazio, John P. Chriseed, P.O. Box 98, Mount Vernon, WA, 98273-0098. Ph: (360) 336-9727; Fax: (360) 424-9520; Email: *john_navazio@alfseed.com*. Breeding for increased pigments in cucurbits, carrots and beets.

Nea, Larry Seminis Vegetable Seeds, 37437 State Highway 16, Woodland, CA, 95695. Ph: (530) 666-0931; Fax: (530) 668-0219. Cucumbers, melons, squash, watermelon.

Ng, Timothy J Dept. Natural Resource Sci., University of Maryland, College Park, MD, 20742-4452. Ph: (301) 405-4345; Fax: (301) 314-9308; Email: *tn5@umail.umd.edu*. Melon breeding and genetics; postharvest physiology; seed germination.

Niemirowicz-Szczytt, Katarzyna Warsaw Ag Univ, Dept Gen & Plt Brdng, ul. Nowoursynowska 166, 02-766 Warsaw, Poland. Ph: (48-22) 843 09 82; Fax: (48-22) 843 90 61; Email: *niemirowicz@alpha.sggw.waw.pl*. Cucumber, melon, winter and summer squash, watermelon - genetics, breeding, tissue culture, biotechnology.

Nuez, Fernando Cat.de Genetica, ETS Ingen. Agron., Univ. Politecnica, Camino de Vera, 14, 46020 Valencia, Spain. Ph: 34 (6) 387-74-21; Fax: 34 (6) 387-74- 29; Email: *fnuez@btc.upv.es*. Genetics and plant breeding.

Oliveira de Paiva, Waldelice EMBRAPA/CNPAT - Caixa Postal 3761, Rua Dra. Sara Mesquita 2270, 60511-110-Fortaleza-Ceara, Brazil. Ph: (085) 299.18.01; Fax: (085) 299.18.03; Email: *Walde@cnpat.embrapa.br*. Research with cucurbit species, especially *Cucumis*, and particularly *Cucumis melo*.

Om, Young-Hyun Natl Horticultural Res Inst, 475 Imok-Dong, Suwon 440-310, Republic of Korea. Ph: 82-0331-290-6171; Fax: 82-0331-295-9548; Email: *omyh@nhri.go.kr*. Breeding of cucurbit vegetables.

Omara, Sadig Khidir Dept. Horticulture, Fac. Agric. Sci., University of Gezira, Wad Medani, P.O. Box 20, Sudan.

Ouyang, Wei United Genetics Seeds Co., 18 W. Hacienda Lane, Woodland, CA, 95695. Ph: (707) 693-6815; Fax: (707) 693-6814; Email: *weiouyang1@yahoo.com*. Squash, watermelon, and melon breeding.

Owens, Ken United Genetics Seeds Co., 8000 Fairview Road, Hollister, CA, 95023. Ph: (831) 636-4882; Fax: (831) 636-4883; Email: *kobreeding@hotmail.com*. Cucumber breeding.

Pachner, Martin Hadikgasse 44/3, A-1140 Wien, Austria. Ph: 43/(0)2272/66280/256; Fax: 43/(0)2272/66280/203; Email: *pachner@ifa-tulln.ac.at*.

Palomares, Gloria Dept Biotecnologia, Univ Politecnica, Camino de Vera, s/n., E-46022 Valencia, Spain. Ph: 34(6)387-7426; Fax: 34(6)387-7429; Email: *gpaloma@btc.upv.es*. Genetic improvement in horticultural plants.

Paris, Harry Dept. Vegetable Crops, A.R.O., Newe Ya'ar Research Ctr, PO Box 1021, Ramat Yishay 30-095, Israel. Ph: 972-4-9894516; Fax: 972-4-9836936; Email: *hsparis@netvision.net.il*. Breeding and genetics of squash and pumpkin.

Pathak, Chandra c/o Nath Sluis Ltd., Nath House, Nath Road, Aurangabad, 431005, India.

Pavlovic, Nenad Center for Vegetable Crops, Karadjordjeva St. 71, 11420 Smederevska Palanka, Yugoslavia. Ph: +381 26 323 170; Fax: +381 26 323 785, Email: *cfvcsp@eunet.yu*.

Peiro Abril, José Luis Apartado de Correos no. 2, E 04720 Aguadulce, Almeria, Spain. Ph: +34 950 34 22

35; Fax: +34 950 34 22 35; Email: *peiroab@larural.es*. Breeding melons, watermelons and cucumber.

Perl-Treves, Rafael Dept. Life Science, Bar-Ilan University, Ramat-Gan, Israel 52900. Ph: 972-3-5318249; Fax: 972-3-5351824; Email: *perl@brosh.cc.biu.ac.il*.

Peter, K.V. Natl. Research Ctr for Spices, ICAR, Post Bag No. 1701, Marikunnu P.O., Calicut - 673 012, Kerala, India. Ph: 011-91-495-258457.

Peterson, Paul S. Plant Pest Diagnostic Center, 3294 Meadowview Road, Sacramento, CA, 95832-1448. Ph: (916) 262-1139; Fax: (916) 262-1190; Email: *ppeterso@cdfa.ca.gov*. Laboratory germination and seed quality assessment.

Picard, Florence Vilmorin, Route du Manoir, 49 250 La Menitre, France. Email: *vilmorin01@brettcomp.com*.

Pitrat, Michel INRA, Domaine St. Maurice, BP 94, 84143 Montfavet cedex, France. Ph: (33) 90 31 63 30; Fax: (33) 90 31 63 98; Email: *Michel.Pitrat@avignon.inra.fr*. Melon, disease resistance, mutants, genetic map.

Poostchi, Iraj 97 St. Marks Road, Henley-on-Thames RG9 1LP, England. Ph: (01491) 574959; Fax: (10491) 574500. Breeding cantaloupes, melons and watermelons.

Poulos, Jean M. Asgrow Italia, Veg. Seeds Srl, Pontinia Research Station, C.P. 110-04014 Pontinia, Italy. Ph: 39(0)773 848549; Fax: 39(0)773 848548; Email: *jpoulos@svseeds.nl*.

Price, E. Glen Sugar Creek Seed, Inc., P.O. Box 508, Hinton, OK, 73047. Ph: (405) 542-3920; Fax: (405) 542-3921; Email: *SGRCRKSD@hintonet.net*. Seedless watermelon; polyploidy, genetics, breeding, cytogenetics.

Provvidenti, Rosario Cornell Univ., Dept. Plant Pathology, NY State Agric. Experiment Sta., Geneva, NY, 14456-0462. Ph: (315) 787-2316; Fax: (315) 787- 2389; Email: *rp13@cornell.edu*. Breeding & genetics of resistance to viral diseases of cucumber, squash, melon, watermelon & other cucurbits.

Ramirez, Pilar CIBCM, Universidad de Costa Rica, San Jose, Costa Rica. Ph: 506-253-5661 x3496; Fax: 506-224-6749; Email: *pramirez@cariari.ucr.ac.cr*. Viruses in cucurbits, production of resistant transgenic plants.

Ray, Dennis Department of Plant Sciences, University of Arizona, Tucson, AZ, 85721. Ph: (520) 621-7612; Fax: (520) 621-7186; Email: *dtray@u.arizona.edu*. Genetics and cytogenetics of *Cucumis melo* and *Citrullus* spp.

Reiten, Joel Territorial Seed Co., P.O. Box 157, Cottage Grove, OR, 97424. Ph: (541) 942-9547; Fax: (541) 942-9881; Email: *tsc@ordata.com*. Bacterial wilt resistance, as well as virus resistance obtained through traditional breeding methods.

Reuling, Gerhard T.M. Nunhems Zaden B.V., P.O. Box 4005, 6080 AA Haelen, The Netherlands. Ph: 0475-599222; Fax: 0475-599223. Cucumber breeding.

Rhodes, Bill B. Clemson Univ./Horticulture, Poole Agricultural Center, Clemson, SC, 29634-0375. Ph: (864) 656-0410; Fax: (864) 656-4960; Email: *BRhodes@clemson.edu*. Watermelon genetics, breeding, micropropagation, disease resistance, male sterility, triploids.

Robinson, R.W. Dept. Hort. Sci., New York State AES, Hedrick Hall, Geneva, NY, 14456-0462. Ph: (315) 787-2237; Fax: (315) 787-2397; Email: *rwr1@cornell.edu*. Breeding and genetics of cucurbits.

Robledo, Claude Seminis - Recherch France, Mas de Rouzel - Chemin des Canaux, 30900 Nimes, France. Ph: 33(0)4.66.38.79.80; Fax: 33(0)4.66.38.79.81; Email: *crobledo@svseeds.nl*. Melon breeding.

Roig, Luis A. Departamental Biotechnology, ETS Ingen. Politec., Camino de Vera 14, 46022 - Valencia, Spain. Ph: 34(6) 3877424; Fax: 34(6) 3877429.

Rorem, Kent Seminis Vegetable Seeds, P.O. Box 667, Arvin, CA, 93203. Ph: (805) 854-2390; Fax: (805) 854-4379. Melon and watermelon breeding and disease resistance.

Saito, Takeo National Research Institute, Veg., Orn. Plants & Tea, Ano, Mie 514-2392, Japan. Ph: +81-59-268-1331; Fax: +81-59-268-1339; Email: *romario@nivot.affrc.go.jp*. Breeding melons resistant to diseases and insects; use of DNA markers for melon breeding.

Sanghani, Amul Unicorn Agrotech Ltd., 1-7-139/3, S.D. Road, Hyderabad, A.p., India 500 003. Ph: +91 40 7811554; Fax: +91 40 7842399; Email: *uniagro@hd1.vsnl.net.in*.

Sarfatti, Matti Hazera Ltd., Research Dept., Mivhor, M.P., Lakhish Daram 79354, Israel.

Schultheis, Jonathan R. Dept. Horticulture, 264 Kilgore Hall, North Carolina St. University, Raleigh, NC, 27695-7609. Ph: (919) 515-3131; Fax: (919) 515- 7747; Email: *jonathan_schultheis@ncsu.edu*. Cultural management of cucurbits; plant spacing, establishment, nutrition, pollination & cultivar evaluation.

Shetty, Nischit V. Asgrow Vegetable Seeds, 432 TyTy Omega Road, Tifton, GA, 31794.

Shifriss, Oved 21 Walter Avenue, Highland Park, NJ, 08904-1709. Precocious pigmentation in *Cucurbita*.

Simon, Philipp W. USDA/ARS-Veg Crops, Dept. Hort., Univ. Wisconsin, 1575 Linden Dr., Madison, WI, 53706. Ph: (608) 262-1248; Fax: (608) 262-4743; Email: *psimon@facstaff.wisc.edu*. Breeding and genetics.

Sipeyre, Bruno Mas de Rouzel, Chemin des Canaux, 30900 Nimes, France. Ph: 66.84.21.32; Fax: 66.38.09.42.

Skirvin, Robert M. Univ. Illinois, Dept. Horticulture, 258 PABL, 1201 Gregory Dr., Urbana, IL, 61801. Ph: (217) 333-1530; Fax: (217) 333-4777; Email: *skirvin@uiuc.edu*. Micropropagation; somaclonal variation.

Staub, Jack E. USDA-ARS, Dept. Horticulture, Univ. Wisconsin, Madison, WI, 53706-1590. Ph: (608) 262-0028; Fax: (608) 262-4743; Email: *jestaub@facstaff.wisc.edu*. Cucumber breeding & genetics, physiology, biochemical genetic markers, evolution, environmental stress.

Stephenson, Andrew G. 208 Mueller Lab, Penn State University, University Park, PA, 16802. Ph: (814) 863-1553; Fax: (814) 865-9131; Email: *as4@psu.edu*.

Stevens, M. Allen Seminis Vegetable Seeds, Inc., 37437 State Highway 16, Woodland, CA, 95695. Ph: (530) 666-0931; Fax: (530) 668-0219. Direction of research.

Stravato, Vittorio Mario via Carlo Levi n. 18, 04022 Fondi -Latina, Italy. Ph: 771-510729; Fax: 771-555036; Email: *sumas@tiscalinet.it*. Disease resistance in Cucurbitaceae species.

Summers, William L. Iowa State University, Dept. Horticulture, Rm. 251, Ames, IA, 50011-1100. Ph: (515) 294-1978; Fax: (515) 294-0730; Email: *summers@iastate.edu*. Genetic improvement of watermelon

Sušić, Zoran Inst. Srbija - Ctr Vegetable Crops, Karadjordjeva St. 71, 11420 Smederevska Palanka, F.R. Yugoslavia. Ph: +381-26-323-170; Fax: +381-26-323- 785; Email: *cfvcsp@eunet.yu*. Genetics and breeding of *Cucurbita* species; cucumber breeding.

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Walters, Terrence The Cucurbit Network, 11901 Old Cutler Road, Miami, FL, 33156-4242. Ph: (305) 667-3800; Fax: (305) 661-5984; Email: *walters@servax.fiu.edu*. Communication via The Cucurbit Network; the whole family Cucurbitaceae.

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Covenant and By-Laws of the Cucurbit Genetics Cooperative

ARTICLE I. Organization and Purposes

The Cucurbit Genetics Cooperative is an informal, unincorporated scientific society (hereinafter designated "CGC") organized without capital stock and intended not for business or profit but for the advancement of science and education in the field of genetics of cucurbits (Family: Cucurbitaceae). Its purposes include the following: to serve as a clearing house for scientists of the world interested in the genetics and breeding of cucurbits, to serve as a medium of exchange for information and materials of mutual interest, to assist in the publication of studies in the aforementioned field, and to accept and administer funds for the purposes indicated.

ARTICLE II. Membership and Dues

1. The membership of the CGC shall consist solely of active members; an active member is defined as any person who is actively interested in genetics and breeding of cucurbits and who pays biennial dues. Memberships are arranged by correspondence with the Chairman of the Coordinating Committee.
2. The amount of biennial dues shall be proposed by the Coordinating Committee and fixed, subject to approval at the Annual Meeting of the CGC. The amount of biennial dues shall remain constant until such time that the Coordinating Committee estimates that a change is necessary in order to compensate for a fund balance deemed excessive or inadequate to meet costs of the CGC.
3. Members who fail to pay their current biennial dues within the first six months of the biennium are dropped from active membership. Such members may be reinstated upon payment of the respective dues.

ARTICLE III. Committees

1. The Coordinating Committee shall govern policies and activities of the CGC. It shall consist of six members elected in order to represent areas of interest and importance in the field. The Coordinating Committee shall select its Chairman, who shall serve as a spokesman of the CGC, as well as its Secretary and Treasurer.
2. The Gene List Committee, consisting of at least five members, shall be responsible for formulating rules regulating the naming and symbolizing of genes, chromosomal alterations, or other hereditary modifications of the cucurbits. It shall record all newly reported mutations and periodically report lists of them in the Report of the CGC. It shall keep a record of all information pertaining to cucurbit linkages and periodically issue revised linkage maps in the Report of the CGC. Each committee member shall be responsible for genes and linkages of one of the following groups: cucumber, *Cucurbita* spp., muskmelon, watermelon, and other genera and species.
3. Other committees may be selected by the Coordinating Committee as the need for fulfilling other functions arises.

ARTICLE IV. Election and Appointment of Committees

1. The Chairman will serve an indefinite term while other members of the Coordinating Committee shall be elected for ten-year terms, replacement of a single retiring member taking place every other year. Election of a new member shall take place as follows: A Nominating Committee of three members shall

be appointed by the Coordinating Committee. The aforesaid Nominating Committee shall nominate candidates for an anticipated opening on the Coordinating Committee, the number of nominees being at their discretion. The nominations shall be announced and election held by open ballot at the Annual Meeting of the CGC. The nominee receiving the highest number of votes shall be declared elected. The newly elected member shall take office immediately.

2. In the event of death or retirement of a member of the Coordinating Committee before the expiration of his/her term, he/she shall be replaced by an appointee of the Coordinating Committee.
3. Members of other committees shall be appointed by the Coordinating Committee.

ARTICLE V. Publications

1. One of the primary functions of the CGC shall be to issue an Annual Report each year. The Annual Report shall contain sections in which research results and information concerning the exchange of stocks can be published. It shall also contain the annual financial statement. Revised membership lists and other useful information shall be issued periodically. The Editor shall be appointed by the Coordinating Committee and shall retain office for as many years as the Coordinating Committee deems appropriate.
2. Payment of biennial dues shall entitle each member to a copy of the Annual Report, newsletters, and any other duplicated information intended for distribution to the membership. The aforementioned publications shall not be sent to members who are in arrears in the payment of dues. Back numbers of the Annual Report, available for at least the most recent five years, shall be sold to active members at a rate determined by the Coordinating Committee.

ARTICLE VI. Meetings

An Annual Meeting shall be held at such time and place as determined by the Coordinating Committee. Members shall be notified of time and place of meetings by notices in the Annual Report or by notices mailed not less than one month prior to the meeting. A financial report and information on enrollment of members shall be presented at the Annual Meeting. Other business of the Annual Meeting may include topics of agenda selected by the Coordinating Committee or any items that members may wish to present.

ARTICLE VII. Fiscal Year

The fiscal year of the CGC shall end on December 31.

ARTICLE VIII. Amendments

These By-Laws may be amended by simple majority of members voting by mail ballot, provided a copy of the proposed amendments has been mailed to all the active members of the CGC at least one month previous to the balloting deadline.

ARTICLE IX. General Prohibitions

Notwithstanding any provisions of the By-Laws or any document that might be susceptible to a contrary interpretation:

1. The CGC shall be organized and operated exclusively for scientific and educational purposes.
2. No part of the net earnings of the CGC shall or may under any circumstances inure to the benefit of any individual.
3. No part of the activities of the CGC shall consist of carrying on propaganda or otherwise attempting to influence legislation of any political unit.
4. The CGC shall not participate in, or intervene in (including the publishing or distribution of statements), any political campaign on behalf of a candidate for public office.
5. The CGC shall not be organized or operated for profit.
6. The CGC shall not:
    a. lend any part of its income or corpus without the receipt of adequate security and a reasonable rate of interest to;
    b. pay any compensation in excess of a reasonable allowance for salaries or other compensation for personal services rendered to;
    c. make any part of its services available on a preferential basis to;
    d. make any purchase of securities or any other property, for more than adequate consideration in money's worth from;
    e. sell any securities or other property for less than adequate consideration in money or money's worth; or
    f. engage in any other transactions which result in a substantial diversion of income or corpus to any officer, member of the Coordinating Committee, or substantial contributor to the CGC.

The prohibitions contained in this subsection (6) do not mean to imply that the CGC may make such loans, payments, sales, or purchases to anyone else, unless authority be given or implied by other provisions of the By- Laws.

ARTICLE X. Distribution on Dissolution

Upon dissolution of the CGC, the Coordinating Committee shall distribute the assets and accrued income to one or more scientific organizations as determined by the Committee, but which organization or organizations shall meet the limitations prescribed in sections 1-6 of Article IX.